JOURNAL OF THE
NATIONAL CANCER INSTITUTE

VOLUME 7 DECEMBER 1946 NUMBER 3

HYPERVOLEMIA SECONDARY TO GRAFTED GRANULOSA-CELL TUMOR[1] [2]

By J. FURTH, *professor of pathology*, and H. SOBEL, *research fellow of the David, Josephine, and Winfield Baird Foundation, Department of Pathology, Cornell University Medical College*[3]

Transplantation studies of granulosa-cell tumors of the ovary disclosed some remarkable secondary changes probably due to sustained massive secretion of products of these cells. Some of these were the known effects of estrogenic hormones, the normal steroid product of the granulosa cells. But whether estrogenic changes were present or not, there was often a great enlargement of the liver, less often of the spleen and adrenals, because of cavernous dilatation of the sinusoids that were engorged with blood (*1, 2*). This condition led us to make blood-volume determinations which disclosed a tremendous increase in blood volume in mice bearing granulosa-cell tumors and an absence of a similar change in mice bearing other tumors.

MATERIALS AND METHODS

The origin and characteristics of strains I, III, and V of transplantable granulosa-cell tumors used in the present study will be more fully described. The subcutaneous grafts were made in the usual manner. When the blood-volume determinations were made, strain I was in its fifth, sixth, and seventh passage, strain III in its sixth, and strain V in its third and fourth.

All ovarian tumors were induced in F_1 hybrids of the inbred Ak and Rf stocks, and all transplantations were carried out in the same stock. The normal control mice were members of the same stock and were of approximately the same age and weight as the tumor-bearing mice. The leukemia used in this study was transplanted in the same F_1 stock, the lung tumor in the Ak, and the hemangioma in the Rf stock. Two mammary-gland tumors were spontaneous, and three were transmitted, both in mice of the C3H stock. The mice were killed at an advanced stage of the disease when it was thought that they would live no longer than a few days. This condition occurred at from 40 to 240 days after implantation of tumor fragments. The grafted tumors weighed between 3 and 13 gm. (9.7 to 31 percent of body weight).

The control mice had tumors as follows: mammary-gland tumors,[4] leukemia, hemangioma, and lung tumors. These tumors weighed between 2 and 20 gm. (6 to 41 percent of body weight).

The postulates for blood-volume determinations and available methods were adequately summarized by Kennedy and Millikan (*3*). We found no data on blood-volume determinations in animals as small as mice, with one exception reported recently by Taylor (*4*), who found that the blood volume of normal mice was about 5 percent of the body weight, as determined by decapitation, squeezing, and washing out as much blood as possible. Taylor noted that the values for blood volume of mice bearing transplanted tumors was 10 to 20 percent higher than in normal mice.

Two procedures for blood-volume determinations were adapted by us for mice: (1) Exsanguination followed by washing out of as many red blood cells as possible; and (2) the Evans blue dye (T-1824) technique. Neither of these is entirely satisfactory, and attempts are being made to improve them.

EXSANGUINATION-PERFUSION TECHNIQUE

The mouse is weighed and anesthetized by intraperitoneal injection of nembutal (about 0.01 cc. of a 1.6-percent solution per gram of body weight). When under light anesthesia, 0.05 cc. of the 1-percent heparin solution is injected into the tail vein, the needle being fastened to the tail by means of adhesive tape, the syringe remaining attached to it. The animal is fastened on the left upper corner of a board, its neck is greased slightly with vaseline, as is the grooved corner of the board; the jugular vein is exposed, the region

[1] Received for publication September 14, 1946.
[2] These investigations have been supported by the Donner Foundation, the Anna Fuller Fund, the National Advisory Cancer Council, and the Jane Coffin Childs Memorial Fund for Medical Research.
[3] The skillful technical assistance of Misses Lucille Wolf and Katie Cobbs is gratefully acknowledged. All photographs were taken by R. J. Oehrig, whose work is also acknowledged.

[4] Obtained through the kindness of Drs. C. J. Lynch and Helene Toolan.

726906—47——1

lightly greased with vaseline, the vein is then cut, and sample 1 of the blood is collected in a small crucible chilled with ice. When the blood flow ceases, sample 1 is transferred to a hematocrit tube, the heparin-containing syringe is removed, and isotonic sodium citrate (1.6 percent) is injected slowly until the perfusion fluid (sample 2) is colorless. Both samples are centrifuged, and in this manner the following values are obtained: (1) Red cell-plasma ratio; (2) cell volume in sample 1; (3) cell volume in sample 2. The sum of (2) and (3) gives the total amount of removable blood cells. After deducting from the plasma volume of the hematocrit the estimated proportion of injected heparin, the total-plasma volume is calculated in the usual manner.

This technique was later modified by cutting the carotid artery instead of the jugular vein. Before this is done, a loose ligature is placed around the artery just above the clavicle, the artery is cut cephalad, and the ligature tied after exsanguination to facilitate perfusion. By this procedure, a larger volume of the first sample, useful for chemical and other studies, is obtained. Before the perfusion is concluded, the pelvis and lower extremities of the animal are raised and gently shaken without detaching the syringe, and perfusion is renewed. The procedure is repeated several times. When the fluid is colorless, the inferior vena cava is cut above the diaphragm, and perfusion fluid is collected from the pleural cavity. Occasionally the needle becomes detached from the tail vein, but perfusion can be continued through the left ventricle of the heart.

Dye Technique

After the injection of heparin into the tail vein of the anesthetized animal, as already described, the syringe is removed without withdrawing the needle; and a second syringe containing 0.150 to 0.300 percent Evans blue (T-1824) solution in saline is inserted, 0.20 cc. is injected, the original syringe replaced, and an additional 0.05 cc. of heparin introduced. The needle is removed, and the needle-puncture wound is sealed with adhesive tape. There is a very slight loss of dye from the injured vein during this procedure. The jugular vein or carotid artery is exposed, and the animal is bled at precisely 6 minutes from the moment of completion of the injection. This sampling time was arbitrarily chosen on the basis of data of others in the literature, and it has not been established that this is optimal for the mouse and that it is the same for normal and hypervolemic mice.

Twenty-hundredths of a cubic centimeter of plasma sample is added to 3.80 cc. of a 5-percent sodium citrate solution, and the optical density measured with the 650μ filter in the Fisher electrophotometer. A standard curve is prepared by mixing 0.2 cc. of pooled plasma of normal mice with 3.60 cc. of 5-percent sodium citrate and varying amounts of dye in 0.20 cc. saline.

Twenty-hundredths of a cubic centimeter is subtracted from the calculated plasma volume. The blood-cell and total blood volume are then figured on the basis of the centrifuged hematocrit value.

RESULTS

The results of blood-volume determinations made by the exsanguination-perfusion technique on 12 normal mice, on 24 mice bearing subcutaneous grafts, and on 10 mice bearing other tumors are summarized in table 1. The blood-volume values for normal mice varied between 3.5 and 6.6 percent of body weight (1.3 to 2.1 cc.), and those of mice bearing ovarian tumors between 7.3 and 35.8 percent (2.0 to 17.2 cc.). The blood volume of mice with tumors other than those of granulosa cells was not significantly greater than that of normal mice. These figures indicate an approximately onefold to fivefold increase in blood volume of mice bearing ovarian tumors over that of normal mice.

Table 1.—*Blood-volume determinations by exsanguination-perfusion technique*

Group	Number	Blood volume: Amount: Range	Blood volume: Amount: Average	Blood volume: Percent of body weight: Range	Blood volume: Percent of body weight: Average	Red blood cells in millions: Range	Red blood cells in millions: Average
		Cc.	Cc.				
Mice with granulosa-cell tumors:							
Total [1]	24	2.0–17.2	5.3	7.3–35.8	13.6	1.8–12.8	7.6
With ++–+++ congestion	5	3.4–17.2	7.8	12.3–35.8	18.7	3.8–9.8	6.4
With +–++ congestion	8	2.0–9.4	4.6	7.3–21.3	11.7	4.0–12.8	8.5
With 0–+? congestion	6	2.0–3.8	3.2	7.6–10.8	9.0	5.0–11.7	10.2
Normal mice	12	1.3–2.1	1.5	3.5–6.6	5.2	9.0–12.7	10.2
Mice with other tumors	10	1.2–3.8	2.3	3.7–11.0	6.9	2.2–8.3	6.1

[1] Total number of mice with granulosa-cell tumors. The degree of congestion was determined microscopically in 19, as indicated.

Better information on the magnitude of the blood-volume changes is obtained by dividing the mice into three groups according to the degree of congestive changes in liver and adrenals as ascertained histologically from sections made after perfusion of the animals. The congestive changes in tumor-bearing mice are illustrated in figures 1 to 5. All sections were fixed in Zenker-formol and stained with hematoxylin-eosin solutions. The magnifications are approximate. Figure 1, *A*, *C*, and *D*, show congestive changes in the liver of nontransfused tumor-bearing mice; figure 2, *D*,

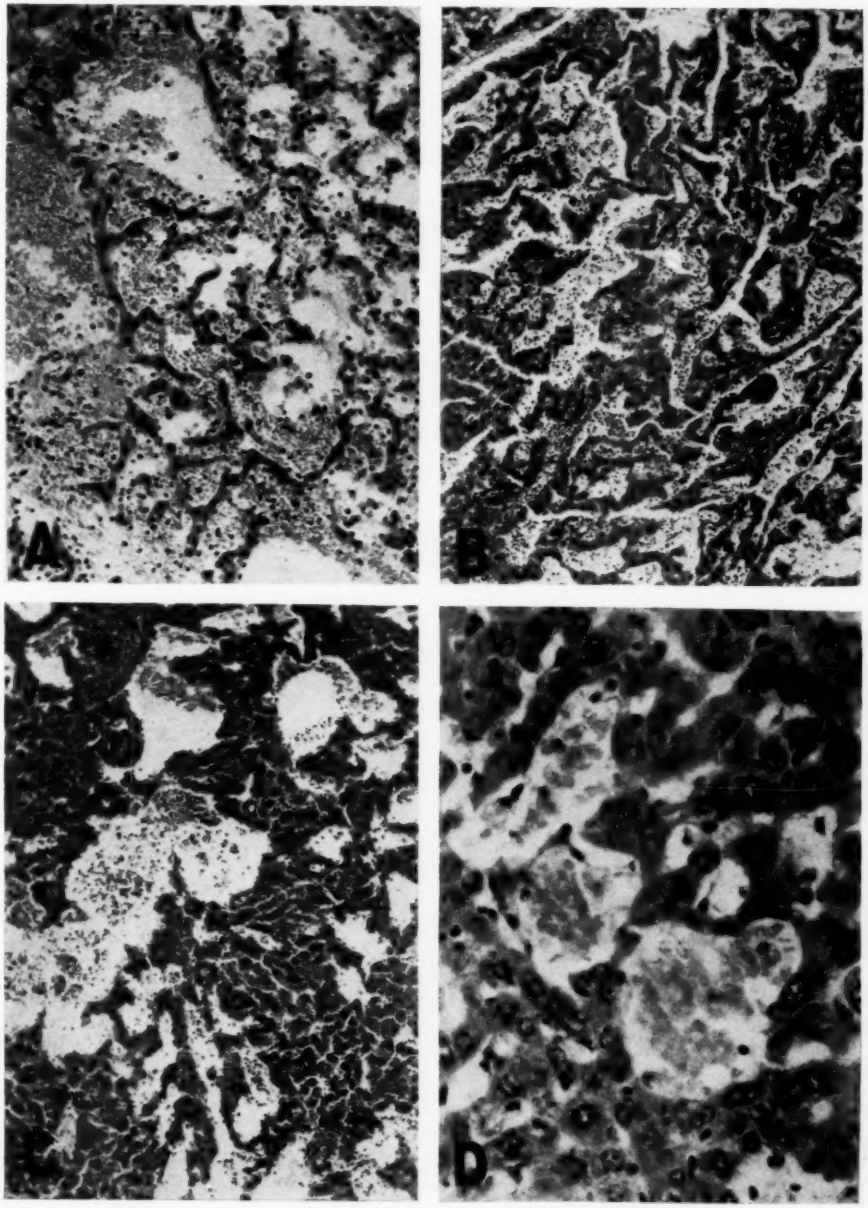

FIGURE 1.—*A*. Liver of mouse Mb 1930, bearing granulosa-cell tumor of strain I, 4 x 2 x 2 cm. Note tremendous engorgement of liver sinusoids. × 90. *B*, Liver of mouse Mc 942, bearing granulosa-cell tumor of strain V, 4.8 x 2.5 x 1.0 cm., fixed and sectioned after exsanguination and perfusion. The section shows collapsed sinusoids and thin, tortuous cords of liver cells. × 90. (Compare with figure 5, *A* and *B*, taken from same mouse.) *C*, Moderate congestion of liver of mouse Mb 1594, bearing granulosa-cell tumor of strain I. Note absence of damage to parenchymal cells. × 180. *D*, Higher magnification of liver of same mouse. × 450.

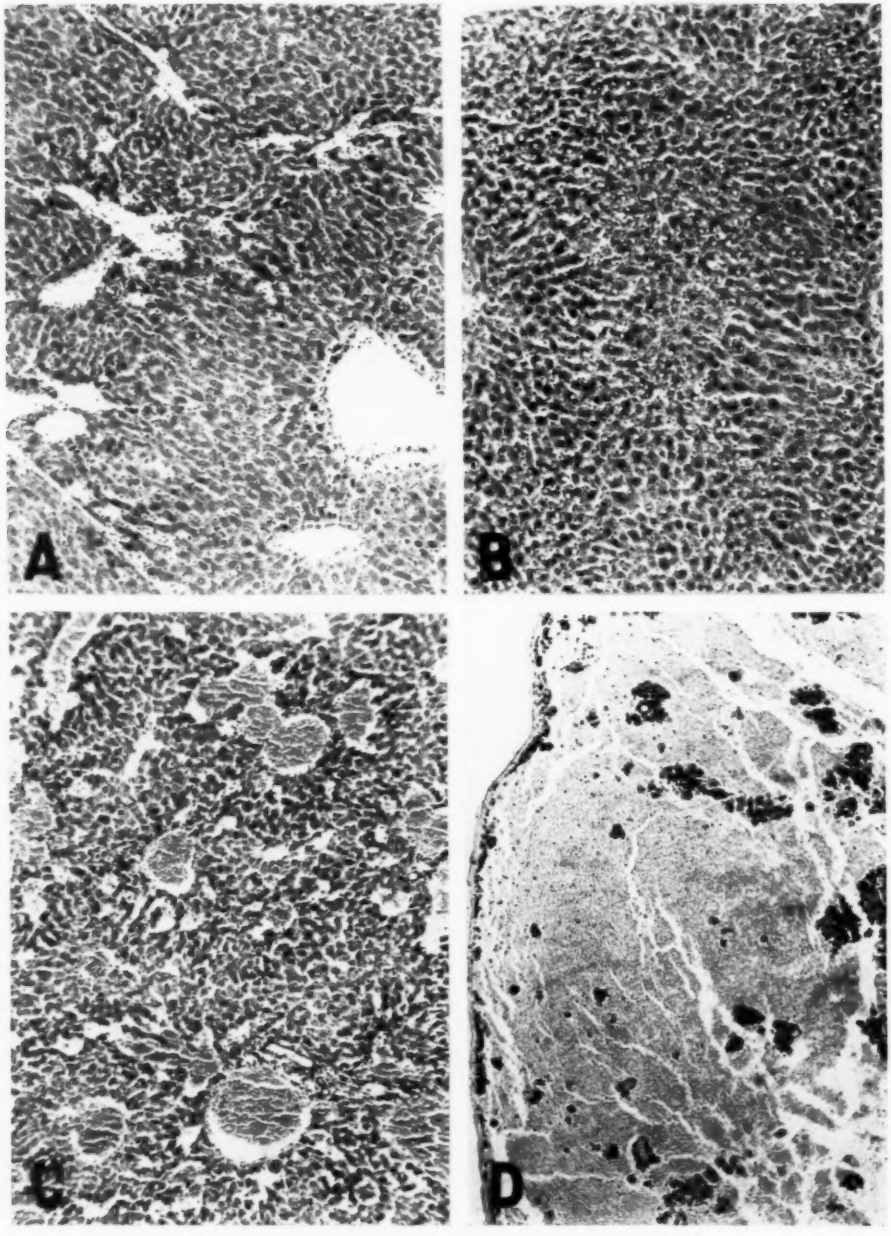

FIGURE 2.—*A*. Liver of mouse Mc 1180, fixed and sectioned after exsanguination and perfusion, showing spotty sinusoidal dilatations. The tumor weighed 9 gm. × 90. *B*, Liver of mouse Mc 1410 bearing a transplantable lung tumor, fixed and sectioned after exsanguination and perfusion. Note complete absence of congestion. × 90. *C*, Liver of mouse Mc 1160. Second intrasplenic passage of granulosa-cell tumor obtained by procedure of Biskind and Biskind (*5*). Note moderate congestion of the liver. × 90. *D*, Section from the spleen of mouse Mc 757, with strain III granulosa-cell tumor. Note advanced hyperemia of the spleen. × 90.

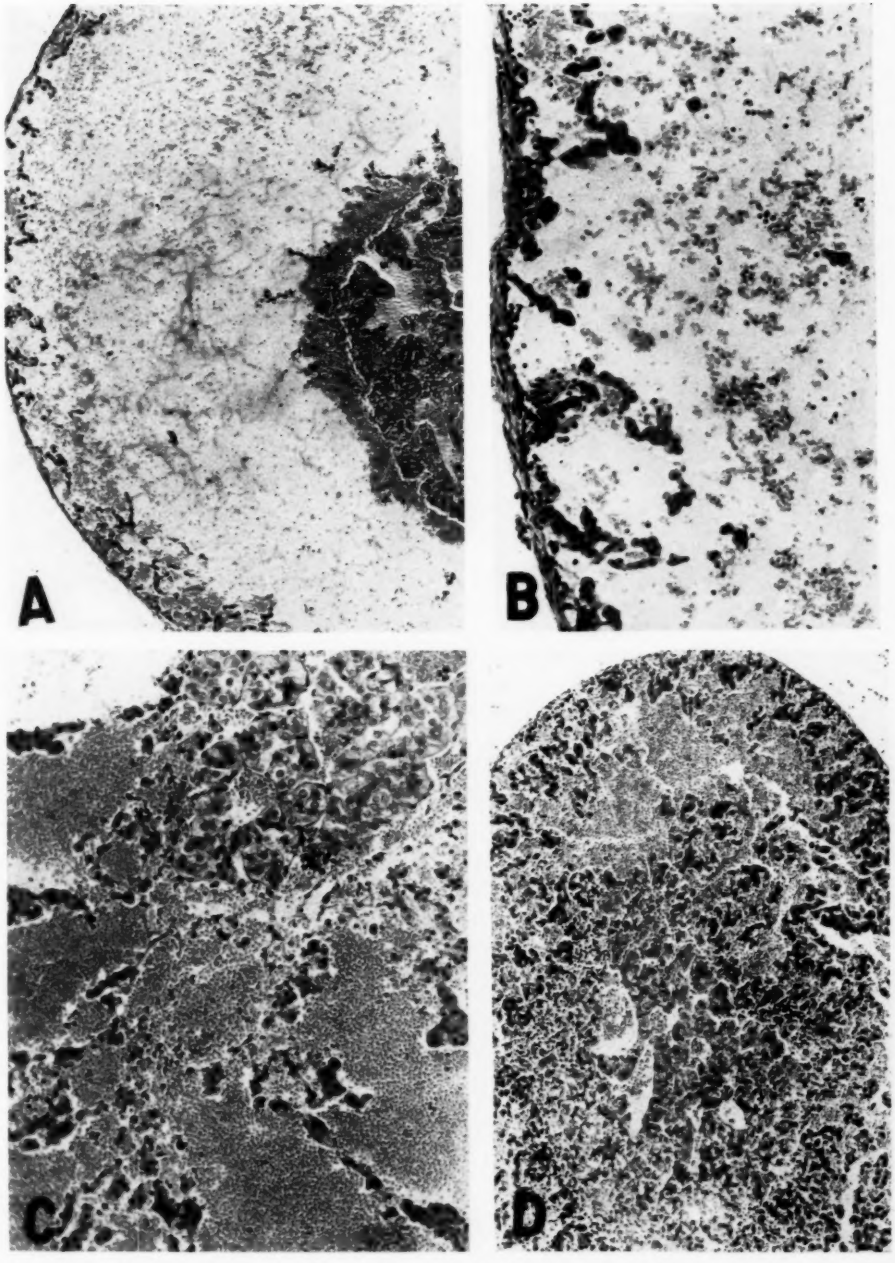

FIGURE 3.—*A*. Advanced congestion of the adrenal of mouse Mc 757, with strain III ovarian tumor. (Compare spleen of this mouse, shown in figure 2, *D*.) Serum precipitate with red blood corpuscles has widened the cortex, which is atrophic. × 90. *B*, Same with higher magnification. × 180. *C*, Adrenal of mouse Mc 1140 with strain III ovarian tumor. Note moderate congestion of adrenal cortex with normal medulla. × 180. *D*, Adrenal of mouse Mc 1143 with strain III ovarian tumor. Note moderate congestion of the adrenal cortex with thinning of the cortical cells and the normal medulla. × 90.

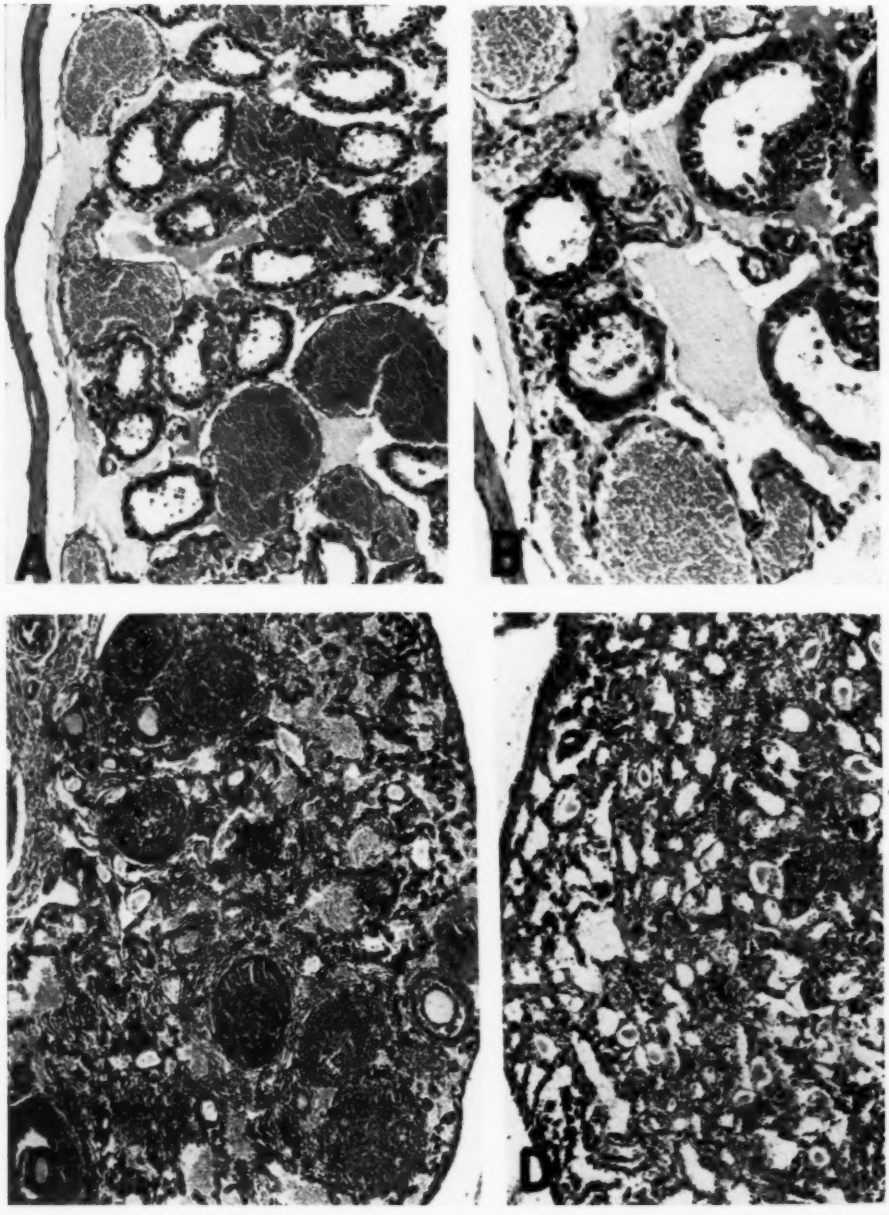

FIGURE 4.—*A*, Testis of mouse Mc 2077 bearing granulosa-cell tumor of strain III. Note enormous congestion of the sinusoids of the capillaries of the testis, which persisted although the animal had been exsanguinated. There were metastases in liver and lung. Note also the advanced atrophy of the spermatogenic tubules. × 90. *B*, Same with higher magnification. × 180. *C*, Ovary of mouse Mc 2045 bearing ovarian tumor strain III, showing moderate congestion of ovary with atrophy of follicles. The liver of this mouse (cf. fig. 5, *D*) had advanced congestion. × 90. *D*, Advanced atrophy of the ovary of mouse Mc 1415 bearing tumor of strain V. Note numerous degenerating ova and slight congestion. × 90.

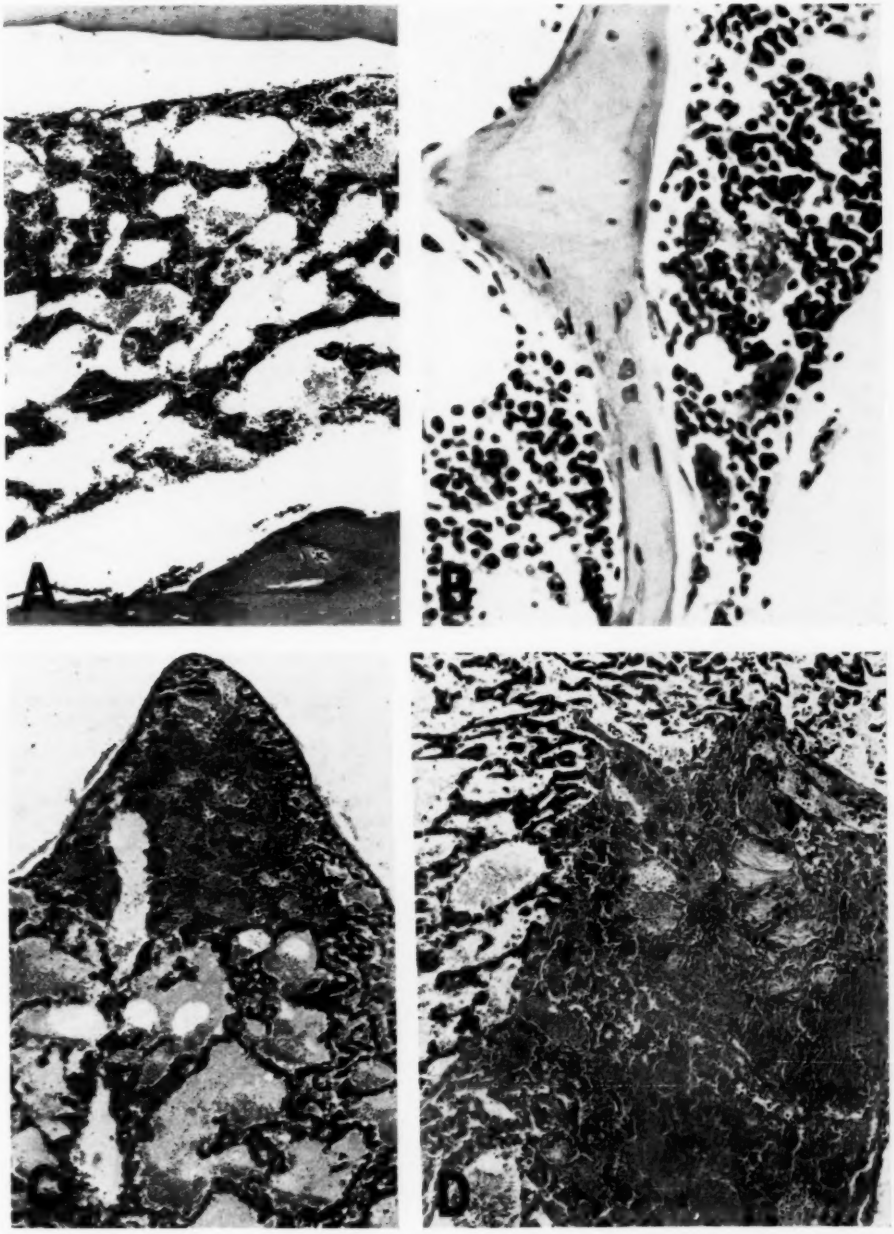

FIGURE 5.—*A*, Bone marrow of mouse Mc 942 bearing ovarian tumor of strain V. There is marked congestion and slight atrophy of the marrow. × 90. *B*, Same with higher magnification. × 450. (Compare figure 1, *B*, from same mouse.) *C*, Marginal infarct of the liver, a complication of the cavernous dilatation of the sinusoids, in mouse Mc 1411 bearing granulosa-cell tumor of strain V. × 90. *D*, Infarct in the liver of mouse Mc 1415, a complication of the cavernous dilatation of the sinusoids. × 90.

shows advanced congestion of spleen, and figure 3, *A–D*, that of the adrenal of similar mice. Even after exsanguination and perfusion, the sections still show distinctly the sinusoidal dilatations, and, in fact, some vessels still contain many red blood corpuscles (figs. 1, *B*; 2, *A*; and 4, *A*).

Table 1 indicates that in the presence of marked congestion the blood volume as determined by this technique is greatly increased. But the table also indicates that the blood volume is distinctly increased when the sections show only slight or questionable congestion (cf. fig. 2, *A*). Hence the dilatation of the vessels appears to be secondary to hypervolemia. Figure 1, *D*, shows the appearance of the liver of a mouse with a marked increase in blood volume, classified in table 1 as +++. This liver was sectioned after exsanguination and perfusion. Figure 2, *A*, shows the liver of a mouse similarly treated, with congestion classified in table 1 as +. For comparison, figure 2, *B*, is given to illustrate the normal liver of a mouse bearing a transplantable lung tumor.

This congestive change in the liver first drew our attention to the possible existence of hypervolemia in granulosa-cell-tumor-bearing mice, in which the liver weighed approximately 4.5 gm., as compared with the normal liver weight of about 1.5 gm. Histologic examinations indicate an increment in blood proportional to the increase in weight of this organ. There is no other microscopic change which could account for an increase in the weight of this organ. Experiments are in progress to determine the relative hypervolemia of different organs, including the liver.

The hyperemia in association with granulosa-cell tumors is very characteristic. It is neither the usual central nor peripheral congestion. It begins in focal areas frequently in proximity to the portal system in which one or two or groups of capillaries become greatly distended (fig. 2, *A*) and soon assume a giant size. It is remarkable that even with moderate hyperemia the liver cells do not appear compressed. In the presence of abnormal congestion, the endothelial cells appear to be increased in number but not in size. As a rule, there is a conspicuous absence of inflammatory reaction. At the very advanced stage of hyperemia, some vessels become thrombosed, and small areas of the liver undergo infarctions (fig. 5, *C* and *D*). Occasionally engorged capillaries beneath the capsule rupture, and the animal dies from peritoneal hemorrhage. In strain I the congestive changes are marked in the liver and are inconspicuous in other organs. In strains III and V, liver, spleen, and adrenals are characteristically engorged with blood, and occasionally the ovaries (fig. 4, *C*) and the testes (fig. 4, *A* and *B*) are moderately congested and the kidneys slightly congested, while all organs above the diaphragm appear normal.

The values obtained with the dye technique (table 2) indicate a rise of blood volume in the ovarian-tumor-bearing mice from one to five times that of normal mice. While the absolute values are in general higher with the dye technique in normal animals (as would be expected), they are so much higher in the granulosa-cell-tumor-bearing animals that it is doubtful whether they are real. Both procedures were applied simultaneously to several animals, and these large differences persisted as indicated by the comparative values shown in table 3. Attempts are being made to determine the cause of this discrepancy.

TABLE 2.—*Blood-volume determinations with the dye (T-1824) technique*

Group	Number	Blood volume			
		Amount		Percent body weight	
		Range	Average	Range	Average
		Cc.	*Cc.*		
Mice with granulosa tumors	10	3.1–20.8	12.8	9.7–54.6	34.3
Normal mice	9	2.4– 4.2	3.4	9.0–12.7	10.9
Mice with other tumors	6	2.0– 6.1	3.2	6.9–12.4	9.0

TABLE 3.—*Simultaneous blood-volume determinations by dye and exsanguination-perfusion techniques*

Group	Dye technique		Exsanguination-perfusion technique	
	Blood volume		Blood volume	
	Amount	Percent body weight	Amount	Percent body weight
	Cubic centimeters		*Cubic centimeters*	
Normal mice:				
Mc 1052	3.31	11.7	1.59	5.7
Mc 1505	3.82	10.0	2.0	5.7
Granulosa-cell-tumor-bearing mice:				
Mc 2074	10.31	23.4	9.4	21.2
Mc 1222	6.69	19.6	3.3	9.6
Mc 1412	12.4	30.2	7.36	17.9
Mc 1415	20.8	54.6	10.9	28.7

The fact remains that huge quantities of blood (4–7 cc.) can be obtained from mice bearing these ovarian tumors by simple exsanguination, with hematocrit values close to or only slightly above or below normal.

The data in table 4 indicate that the total number of red blood cells in hypervolemic mice is enormously increased even though the red-blood-cell count in these mice is below normal.

TABLE 4.—*Data on total number of red cells and on their corpuscular volume in hypervolemic mice*

Blood data	Normal control mice		Hypervolemic granulosa-cell-tumor-bearing mice	
	Range	Average	Range	Average
Blood volume:				
Cubic centimeters	1.28– 2.40	1.59	3.05– 9.36	4.90
Percent body weight	3.5– 7.3	5.6	8.2– 21.6	13.0
Red-cell count, in millions per cubic millimeter	8.55–12.6	10.2	5.79–11.70	8.80
Total red cells in blood, in billions	14.0– 26.0	16.3	24.9– 54.3	40.9
Cells in whole blood (hematocrit value), percent	41.8– 59.3	52.4	24.5– 62.5	41.1
Mean corpuscular volume, cubic μ	40– 63	52	35– 55	45
Red cells per gram of body weight, in millions	380– 790	530	[1] 870–1,260	[1] 1,080

[1] The tumor weight is included in body weight.

For this analysis eight mice with hypervolemia caused by granulosa-cell tumors and eight normal mice were selected at random. The blood-volume values were obtained by the exsanguination-perfusion technique.

In this group of mice, the total number of circulating red cells was about twice that of normal although the number of red blood cells per cubic millimeter (red-blood-cell count) was slightly below normal. Decreases in the mean corpuscular volume indicate that the "anemia" was microcytic. Incidentally, these experiments indicate that without a blood-volume determination the term "anemia" may be meaningless.

The tumor mass plays an insignificant part, if any, in contributing to this hypervolemia since it is absent in other tumors equally well vascularized. A systematic correlation of tumor size and blood volume was not made. A tentative analysis failed, however, to show a direct relationship between tumor size and blood volume. Some animals with large granulosa-cell tumors had smaller blood volumes than did others with smaller tumors. On gross dissection, large quantities of blood were still present in tumors of perfused animals (e. g., fig. 1, *B*; fig. 4, *A* and *B*). Thus it seems that the blood cannot be readily washed out from these grafted tumors which are relatively poor in well-formed arteries and veins and rich in giant capillaries.

DISCUSSION

Much work has been done on alterations in blood volume in man and animals, but little is known about the mechanism of maintenance of blood volume. A reasonable classification of changes in the blood volume has been proposed by Rowntree and associates (*6*), and their term "hypervolemia" for blood-volume increase has found wide acceptance.

It is known that the blood volume is decreased in chronic anemia and dehydration; it is increased in polycythemia and during pregnancy. The increase in blood volume in polycythemia is considered to be secondary to that of red blood cells. While data have been gathered on the part played by endocrine secretions in erythrogenesis (*7*), their influence on blood volume has not been adequately investigated. The cause of the hypervolemia of pregnancy, which amounts to a 60 percent plus (*8*) is not known, and suggestions concerning its possible mechanism include pressure on pelvic vessels by the pregnant uterus with secondary venous stasis, arterio-venous shunt in the vascular bed of the placenta, and secondary changes due to some female sex hormone (*8*). It is known, however, that desoxycorticosterone acetate raises somewhat the blood volume in both man and animals in association with a marked retention of sodium and chloride (*9*).

Many questions remain to be answered. What is the direct cause of the blood-volume increase in mice with granulosa-cell tumors? It appears to be due to some specific secretion of this ovarian tumor, but such a product remains to be demonstated. What is the point of attack of the hypothetical substance? In our experimental hypervolemia, the hematocrit values for red blood cells are normal or slightly above or below normal, while in human polycythemia there is a great increase in red-cell count and cell volume. Furthermore, human polycythemia is not accompanied by an increase in plasma volume while in our experimental hypervolemia both red-cell and plasma volume are raised. In polycythemia produced by injections of cobaltous chloride into rats (*10*), there was an 80-percent increase in blood volume, a 16-percent decrease in plasma volume, and a 192-percent increase in total erythrocyte mass.

In general, our experimental hypervolemia seems similar to but is much greater than that in pregnancy. The hypervolemia produced by desoxycorticosterone acetate is accompanied by sodium retention. Data on salt and water balance in our experimental mice are not yet available. The granulosa-cell tumors are accompanied by massive hyperemia with atrophy of the adrenal cortex, and this condition might be expected to cause hypovolemia rather than hypervolemia unless the tumor itself secretes a hormone similar to that of the adrenal cortex. Excessive amounts of thyroid hormone are stated to be able to cause some blood-volume rise, and myxedema is accompanied by hypovolemia, but deviations from normal in these conditions are slight (*6*).

The most marked sites of congestive changes in mice with hypervolemia are liver, spleen, and adrenals. Congestive changes in organs above the diaphragm are slight, if any.

The blood-forming organs of numerous granulosa-cell-tumor-bearing mice were sectioned, and blood smears of several mice with hypervolemia were made at the time that the red blood cells were counted. This material is the subject of current study. It seems certain that the bone marrow is frequently congested and that it contains the usual hemopoietic cells, most of which are erythroblasts; megakaryocytes are numerous (fig. 5, *A* and *B*). The spleen is the site of advanced erythrogenic metaplasia. The blood smears of three mice with extensive hypervolemia showed no important deviation from normal. The percent of polymorphonuclear leukocytes was 18–43; of eosinophils, 2–6; of lymphocytes, 46–73; and of monocytes, 3–7. The absence of precursors of white blood cells is noteworthy. Platelets appeared to be present in the blood smears in about normal numbers. The white-cell counts of several mice examined were approximately within normal limits.

Blood-pressure measurements were not made. The hearts of these mice are dilated but not distinctly hypertrophied. Determinations of total plasma proteins and albumin of several mice bearing granulosa-cell tumors and having a moderate or marked hypervolemia gave approximately normal values. However, there is frequently hypoglobulinemia and a rise in blood urea nitrogen.

The genesis of hypervolemia in animals bearing granulosa-cell tumors is puzzling. Since the liver is known to metabolize circulating estrogens, it seems possible that either estrogens or their metabolites are the immediate cause of this change. But similar alterations have not been noted in mice given large doses of estrogens. No one, to our knowledge, has tested the effect of administering steadily increasing amounts of estrogens as can be supposed to occur in mice bearing transplanted tumors secreting estrogens. The first transplantable granulosa-cell tumor was described by Strong, Gardner, and Hill (*11*), who did not report a congestive change in their tumor-bearing mice although the tumor secreted estrogens. Furthermore, congestive changes are not produced by some of our transplantable granulosa-cell tumor strains while they are present in mice bearing tumors of strain I with no evidence of hyperestrinization, as well as in mice bearing granulosa-cell tumors of strains III and V, in which there was evidence of hyperestrinization. The latter is indicated by hyperplasia of the uterus and keratinizing metaplasia of the epithelium of the vagina in castrated tumor-bearing females, pronounced atrophy of the testes (fig. 4, *A* and *B*), and of the seminal vesicles in normal adult tumor-bearing males, as compared with their nontumor-bearing siblings.

Recently we noted a moderate congestive change of this type in the liver of mice bearing granulosa-cell growths produced by the method of Biskind and Biskind (*5*) (fig. 3, *C*).

If the primary change is a hyperemia in the liver, a secondary congestion in the portal system might follow, but it is puzzling why the adrenals, which are drained to the systemic circulation, should be as much congested as the liver, while the spleen is less or not at all congested. If the blood-volume increase is the primary process, it remains to be explained why the congestive changes are massive in certain organs as liver and adrenals and are absent in others.

It is possible that the hypothetical substance derived from granulosa cells is related to one which normally influences maintenance of the blood volume, and that the rise is due to an excessive amount of this substance. It is possible that this hypothetical substance is related to the vasodepressor of Shorr, Zweifach, and Furchgott (*12*) and personal communication) and that the phenomenon here described is an unusual disturbance of the vascular homeostatic mechanism caused by an excess of the vasodepressor.

SUMMARY

Determinations made of the blood volume of mice bearing transplantable granulosa-cell tumors indicate a onefold to fivefold rise of blood volume over that of normal mice. There is a secondary cavernous dilatation of the liver and of several other abdominal viscera. Hypervolemia may occur with or without the known estrogenic morphologic changes. The absolute volume of both cells and plasma is increased. This readily reproducible state may offer a means of studying the mechanism of the maintenance of blood volume.

REFERENCES

(*1*) Furth, J., and Boon, M. C.: Liver changes associated with a transplantable granulosa-cell carcinoma in mice. Proc. Soc. Exper. Biol. & Med. 58: 112–114 (1945).

(*2*) Furth, J.: Transplantability of induced granulosa cell tumors and luteoma in mice. Secondary effects of these growths. Proc. Soc. Exper. Biol. & Med. 61: 212–214 (1946).

(*3*) Kennedy, J. A., and Millikan, G. A.: Microblood volume method using blue dye and photocell. J. Physiol. 93: 276–284 (1938).

(*4*) Taylor, A.: Cancer studies. Univ. Texas Publ. p. 95 (1945).

(*5*) Biskind, M. S., and Biskind, R. R.: Development of tumors in the rat ovary after transplantation into the spleen. Proc. Soc. Exper. Biol. & Med. 55: 176–179 (1944).

(*6*)

(*7*)

(*8*)

(*9*)

(*6*) ROWNTREE, L. G., and BROWN, G. E.: The volume of the blood and plasma in health and disease. W. B. Saunders Co., Philadelphia (1929).

(*7*) GORDON, A. D., and CHARIPPER, H. A.: The endocrine system and hemopoiesis. Presented at the New York Acad. Sc., April 19, 1946. (To be published.)

(*8*) THOMSON, K. J., HIRSHEIMER, A., GIBSON, J. G., JR., and EVANS, W. A., JR.: Studies on circulation in pregnancy; blood volume changes in normal pregnant women. Am. J. Obst. & Gynec. 36: 48–59 (1938).

(*9*) CLINTON, M., JR., and THORN, G. W.: Effect of heavy desoxycorticosterone acetate administration on plasma volume and electrolyte balance of normal human subjects. Bull. Johns Hopkins Hosp. 72: 255–264 (1943).

(*10*) STANLEY, A. J., HOPPS, H. C., and HELLBAUM, A. A.: Observations on cobalt polycythemia. I. Studies on the peripheral blood of rats. Proc. Soc. Exper. Biol. & Med. 61: 130–133 (1946).

(*11*) STRONG, L. C., GARDNER, W. U., and HILL, R. T.: Production of estrogenic hormones by transplantable ovarian carcinoma. Endocrinology 21: 268–272 (1937).

(*12*) SHORR, E., ZWEIFACH, B. W., and FURCHGOTT, R. F.: On the occurrence, sites and modes of origin and destruction, of principles affecting the compensatory vascular mechanisms in experimental shock. Science 102: 489–498 (1945).

BLOOD URIC ACID AND PROTEOSE, BODY TEMPERATURE, AND GLOMERULAR CLEARANCE OF RABBITS IMPLANTED WITH THE BROWN-PEARCE CARCINOMA AND TREATED WITH THE POLYSACCHARIDE FROM SERRATIA MARCESCENS [1]

By BENTON B. WESTFALL, *biochemist*, and THELMA B. DUNN, *research fellow in pathology, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

The blood proteose (*1, 2, 3*) has been found to be increased in rabbits (*2, 3*) bearing the Brown-Pearce carcinoma. An increase has likewise been found in the presence of febrile disease, such as pneumonia. The origin of the increased quantity is unknown, although some experiments (*1*) have indicated that in rats bearing cancers, the cancers themselves are the source. No information is available as to the factors concerned in those animals having pneumonia. It had been noted that animals implanted with the carcinoma occasionally showed an inflammatory reaction at the implantation site, with a simultaneous rise in body temperature and blood proteose before the carcinoma had time to establish itself. The work reported herein was carried out to see if elevation of body temperature independent of infection or inflammation would produce a rise in blood proteose. As a febrifacient a single intraperitoneal dose of the polysaccharide isolable from cultures of *Serratia marcescens* (*4, 5*) was used, largely because of its availability and because it gave the opportunity to study simultaneously not only the effect of temperature on blood proteose but also any deleterious effect of the polysaccharide on the tumor. One of the striking changes noted in the blood of several human patients (*4*) given the polysaccharide intramuscularly was the elevation of the blood uric acid as the temperature rose. This change seemed to point to increased cellular destruction or metabolism or altered metabolism of nucleoproteins or purines. Accordingly, it was deemed advisable to supplement the observations on proteose and temperature with a simultaneous study of the blood uric acid. As the work progressed, it was found that the treated animals showed a train of symptoms compatible with the diagnosis of peripheral vascular collapse, and it was thought that this collapse might result in decreased renal clearance of substances from the blood. If such a decrease took place, it could result in a slow increase in the blood concentration of such waste products as uric acid since the available evidence points to the elimination of uric acid or allantoin by glomerular filtration. For this reason, glomerular filtration as measured by creatinine clearance was estimated in all the later work.

The general findings resulting from this study are that with the tolerated dose of polysaccharide there was no significant rise in the blood proteose, a moderate rise in body temperature, a slight but never pronounced rise in blood uric acid, and in those cases where there was a rise in the blood uric acid an accompanying decrease in glomerular filtration.

[1] Received for publication July 30, 1946.

MATERIALS AND METHODS

Since the study was primarily concerned with the behavior of the blood proteose in the animals before and after a single treatment with the polysaccharide [2] in the presence of the carcinoma, it was limited to rabbits previously inoculated with the Brown-Pearce tumor.

Uric acid (*10*), creatinine [3] (*11*), and creatinine clearance were estimated by established procedures. The deproteination procedure for the plasma creatinine was that of Fujita and Iwatake (*12*). Urine collection periods were started and ended by washing the bladders of the rabbits with 2 cc. of 0.3 percent saline three times. Clearances were calculated by the conventional method, and surface area was calculated by the modified Meeh-Rubner formula (*13–15*):

$$\text{S. A.} = 8.9 \times 10^{-1} \times W^{0.7} \times \frac{Nin}{Nob}$$

where the symbols have the usual significance, *Nin* being the figure 0.39, obtained from 20 normal rabbits, i. e., part of those later inoculated with the tumor, housed under our conditions and fed all they would eat of a commercial pellet with weekly supplements of carrots and cabbage.[4] The normal animals tended to be a little fat; and most of the experimental animals were losing weight because of the tumor, consequently the correction for the nutritional state made the quotient *Nin/Nob* always greater than 1. The rabbits were on a uniform diet and were of about the same initial weight so that in no case did their surface areas differ by more than 8 percent.[5]

[2] The polysaccharide solution, made available by Dr. M. J. Shear, of this Institute, was an aliquot of a potent preparation (*4, 5*) furnished by him to several investigators (*6–9*).

[3] Of the creatinine in stock, supplied by three firms, all samples on analysis were found too low in nitrogen content; but much more serious, as far as this work was concerned, was the fact that all contained sufficient pyrogenic material to produce, at the dose level used in 15 to 20 cc. distilled water, a postinjection rise in body temperature of from 0.2° to 0.5°. Both of these defects were remedied by two recrystallizations by way of the zinc chloride salt.

[4] No attempt was made to promote or maintain any other than the normal urine flow in these animals. Comparison of the renal clearances calculated herein with values for rabbits from the literature (*16*) indicates that at similar levels of urine flow the former are considerably greater, probably because of the stimulating effect on urine excretion of the dietary supplement of carrots (*17*).

[5] Since the work of Stewart (*18*), it has been generally accepted that kidney weight, and by inference functional kidney tissue, are more nearly proportional to surface area than to any other crude measure of body size such as weight or length. In no case is this correct in any exact sense for all species or for any one species throughout its life span (*19*). An equation of the sort

Rectal temperatures were recorded with a calibrated clinical thermometer, allowing a minimum of 3 minutes for temperature equilibration. The temperature of the animal room (26.5° C.) varied by no more than ±1° during the course of the usual experiment of 24 to 72 hours' duration. On the rare occasions when the animals were transferred to the laboratory at 21°, their rectal temperatures customarily showed a small decrease (0.2° to 0.3°) after being subjected to this environmental temperature for some time. The rabbits were trained to lie quietly during the manipulations needed for obtaining blood and urine samples and for recording body temperatures. The labile thermoregulatory mechanism (*19, 20, 21*) of these animals made this training necessary since severe struggling by itself produced a slight elevation of temperature. Rabbits are reputed to show a mild pyrexia following ingestion of food. For this reason, food was withheld 3 hours before the start of the experiment, but water was available at all times. The animals with one exception neither ate nor drank for 20 hours following the administration of the polysaccharide.

Blood plasma proteose was estimated polarographically essentially according to the procedure of Winzler and Burk (*1*), except that filtrates from oxalated plasma instead of whole blood were used. The use of plasma rather than serum uniformly gave lower polarographic waves. The peripheral vascular collapse shown as part of the reaction of the subject to the polysaccharide made the withdrawal of numerous blood samples difficult, so that sampling was confined to the collection of oxalated whole blood at each interval, instead of oxalated whole blood and blood for serum as originally planned.

The rabbits, all adult males, were inoculated intratesticularly at least 21 days previously with the Brown-Pearce carcinoma. Blood was obtained from the ear vein. Cardiac puncture was avoided whenever possible because of the ease with which hemorrhage seemed to occur when the animals were thus traumatized while under the influence of the polysaccharide. To terminate an experiment, they were killed by injection of air into the blood vessels of the ear. Necropsy records were made of any abnormality, the extent of tumor involvement, and amount of hemorrhage. When warranted, tissues were taken for microscopic study. Necropsies were performed on 40 of the animals used in this study. In 21 of these a gross examination only was made, the condition of the thoracic and abdominal organs described, and an estimation made of the extent of the tumor. In 19 animals histologic sections were taken as well. These always included several sections of tumor from various sites, sections from organs in which some effect of the polysaccahride injection might be expected, and any organ in which there was macroscopic evidence of an abnormal condition. For comparison, post mortem studies were made of 5 other rabbits in which the tumor had been established but with no polysaccharide treatment. These control animals were essentially similar to others bearing the Brown-Pearce tumor which have been observed during the years that this tumor has been carried in transplant at the National Cancer Institute. Because of these previous observations and the well-known morphology of this tumor, described in many previous reports, the small number of 5 animals seemed adequate.

developed herein holds reasonably well over the limited size range used, and when applied to the one sex only. Its use makes possible comparison of renal function in animals of somewhat different size and facilitates comparison with values from the literature. In any event, it has no effect on the validity of the conclusions to be drawn regarding one animal when clearances were measured before and after administration of the polysaccharide.

RESULTS

Because the rabbit was known to be three times less sensitive than human beings to bacterial pyrogens (*22*), the exploratory experiments were started using the large dose of 300 micrograms of the polysaccharide per kilogram of body weight at a concentration of 100 micrograms per cubic centimeter of isotonic Ringer's solution. The first rabbit given this dose had only small testicular tumors that had already undergone spontaneous necrosis and reduction in tumor size, and this animal tolerated the dose reasonably well. The second, with extensive, progressing tumors, died in 4 hours in general collapse. By reducing the dose in successive experiments, it was found that the much smaller amount of 5 micrograms/kilogram was that which killed no animals. In general it was found from this part of the study that the amount of the polysaccharide tolerated was in inverse proportion to the amount of viable tumor tissue present and that extratesticular tumor had the most effect on this tolerance, since in no case was there any extensive hemorrhage in the testes attributable to the medication. It was impossible to select two or more rabbits with the same amount of tumor involvement so that the response of any one animal to the polysaccharide was somewhat different from that of any other.

Since this work was completed, Beck and Fisher (*23*) reported their detailed study of the temperature response of normal rabbits to both intravenous and intraperitoneal administration of an aliquot portion of the polysaccharide used for this investigation. They found that the normal rabbit is killed with a much smaller dose per unit of body weight than is the mouse, guinea pig, or dog. Tumor-bearing mice are known to be much less

resistant to the lethal effects of the agent than are normal mice, and intercomparison of our data on rabbits bearing the Brown-Pearce carcinoma with the data of Beck and Fisher indicates that a similar statement applies to cancerous and normal rabbits.

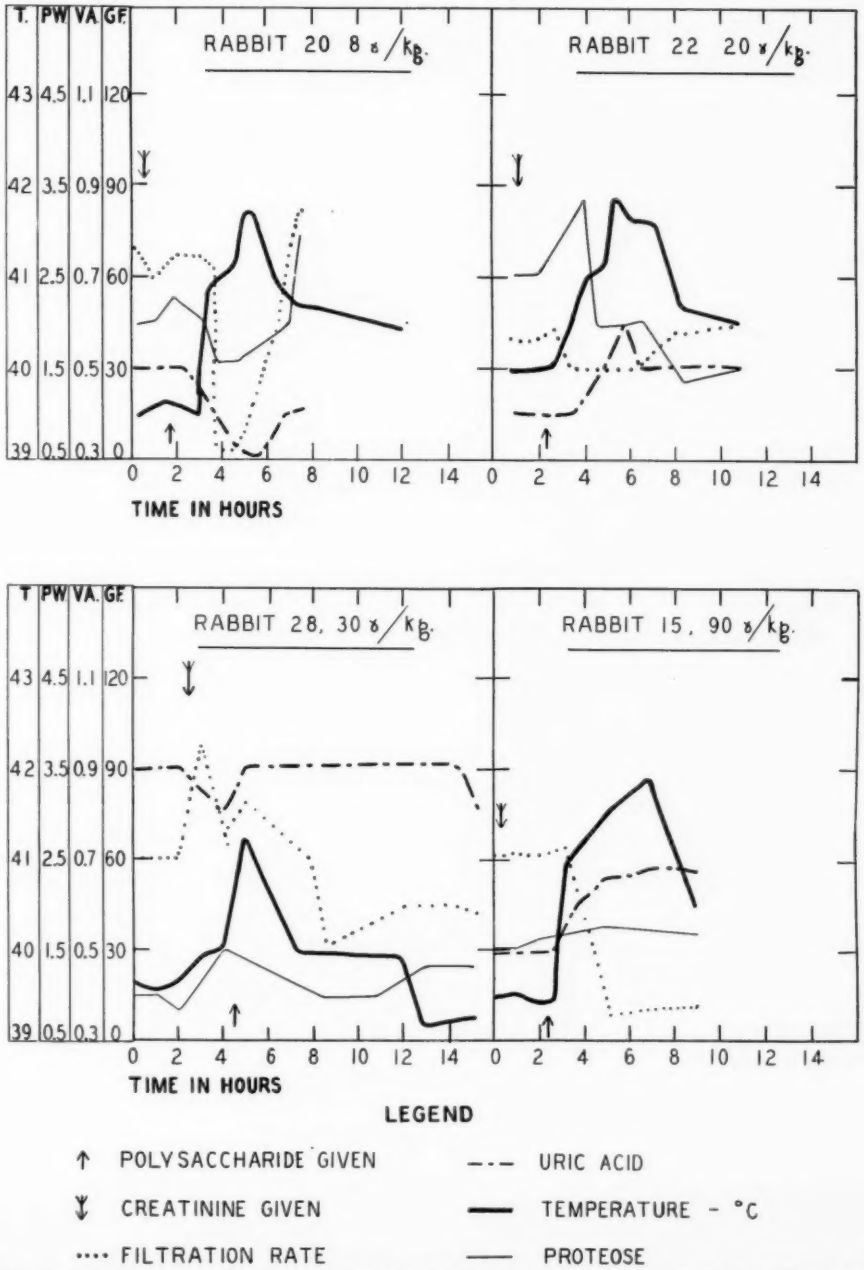

FIGURE 1.—Time course of the behavior of the rectal temperature (T), blood proteose wave (PW), blood uric acid (VA), and glomerular filtration (GF) before and after a single injection of the polysaccharide from *Serratia marcescens*.

Representative results are shown in tables 1 and 2 and in figure 1.

TABLE 1.—*Rectal temperature and blood uric acid of rabbits before and after implantation of the Brown-Pearce tumor*

Rabbit No.	Temperature		Uric acid		Amount of Brown-Pearce tumor involvement
	Initial	After implantation with tumor	Initial	After implantation with tumor	
	°C.	°C.	Milligrams percent	Milligrams percent	
1	39.0	39.7	0.55	0.59	Small.
2	39.1	39.5	.41	.60	Medium [1]; R+ [2].
3	39.0	39.5	.41	.59	Very large; R−.
4	39.3	39.6	.47	.60	Small; R+.
5	39.2	39.0	.41	.94	Very large; R+++.
6	39.0	39.7	.43	.45	Small; R−.
26	39.1	39.2	.47	.79	Small.[1]

[1] Spontaneously necrotic. [2] R, regression noted.

For a few of the rabbits, temperatures and blood-uric-acid levels were recorded before inoculation and after establishment of the Brown-Pearce tumor, as shown in table 1. There was frequently a slight temperature elevation over the normal, particularly if the tumors were large and necrotic. This was likewise true of the blood uric acid. The change in either was not very great and correlated poorly with the extent of tumor metastasis. Since these observations were secondary to the main problem, they were not followed extensively.

Table 2 records in some detail the results on several animals at various dose levels. The data in columns 8 and 9 are rough approximations; thus, "extensive" means from 50 to 200 gm. of tumor tissue present. Animals such as No. 3, given a large dose of 54 micrograms/kilogram and having many tumor metastases, did not live long enough to give a satisfactory experiment. This animal died 6 hours after being given the pyrogen; the body temperature was still at its maximum of 41.9° C., a temperature that had been maintained for 3 hours. Necropsy showed two large testicular tumors (20 gm.), bilateral metastases on the spermatic cord (30 gm.), and numerous tumor nodules in the intraperitoneal, mesenteric, and omental lymph nodes, several large metastases on the diaphragm and nearby chest wall, and congestion of the lungs. The abdomen was filled with blood-stained ascitic fluid, which was judged to be correlated with the circulatory collapse that developed 3 hours before death. Previously no exudate or fluid could be aspirated from the cavity at the time that the intraperitoneal injection of the polysaccharide was given.

TABLE 2.—*Effect of the polysaccharide at various dose levels, rectal temperature, plasma protcose wave, and blood uric acid of the rabbit*

Rabbit No.	Body weight	Dose per kilogram body weight	Maximum rectal temperature rise	Proteose wave change	Blood uric acid		Extent of tumor tissue [1]	Hemorrhage noted at necropsy	Microscopic findings	
					Pre-experimental	Maximum under treatment			Regression	Necrosis and other lesions observed
	Kilograms	γ	°C.		Milligrams percent	Milligrams percent				
8	2.8	215	0.6	0	0.7	1.6	E	+++	−	+++, recent.
9	2.6	110	1.1	−.8	.4	.4	E	+++	−	+
11	2.7	0					E	+	−	++
4	3.8	40	2.3	±.4	.5	.8	VL	0	+	++, coccidiosis in liver.
3	2.8	54	2.8	−2.0	.6	.9	E	+++	−	+++
6	3.5	17	.7	+3.0	.4	.4	VL	0	−	+
13	2.2	9	1.9	0	.3	.4	E	++	−	+
14	3.4	9	1.8	−.7	.6	.4	M	++	+++	+
17	3.0	20	1.2	0	1.0	1.1	L	0	No slides	No slides.
18	2.7	20	1.1	+.5	.7	.8	M	+	do	Do.
20	2.7	8	1.7	+.3	.3	.5	L	+	do	Do.
21	2.2	5	.2	0	1.0	.8	M	+	do	Do.
22	2.7	20	1.9	+.8	.6	.6	N	0	No tumors	"Alarm reaction" effect in thymus.
23	3.7	5	.7	+.8	.4	.5	E	+	No slides	No slides.
24	2.5	5	1.3	.2	.7	.9	E	+	do	Do.
25	2.5	30	1.8	.7	.9	.9	M	0	do	Do.

[1] E, extensive; VL, very little; M, moderate amount; L, little; N, none.

Most animals tolerated the lowest dose of 5 micrograms/kilogram reasonably well, but this dose produced no hemorrhage and usually little change in temperature. The maximum rise observed with this quantity was that shown by rabbit 16, not included in the tables because, although it survived, during the period of maximum temperatures (40.6° C.), it was in such a state of collapse as to prevent adequate blood sampling. Ventricular puncture was not resorted to in most of such cases because the ensuing traumatic hemorrhage made difficult the interpretation of hemor-

rhage due to the agent. Rabbit 11 is included in the tables, although not given the polysaccharide, in order to illustrate the finding that there is some spontaneous hemorrhage in these tumors, especially in the metastases involving the abdominal nodes.

Figure 1 gives the time course of events on two of the rabbits from table 2 and two not included in that table. Rabbit 22 was inoculated with the tumor 29 days before the start of the experiment and at necropsy was found to have very little tumor tissue present. It was given 20 micrograms/kilogram of the diluted polysaccharide between periods 1 and 2. It was noted that this animal had a slightly elevated temperature at the start of the experiment before administration of any pyrogen. There was a slight but hardly significant rise in the blood uric acid after injection of the polysaccharide. Insofar as there was a rise, it could be explained by the drop in the excretion by the kidney. If uric acid were formed at a constant rate and excreted by the kidney solely, all that would be needed to produce this slight rise would be the decrease in kidney extraction as shown by the drop from 38 to 31 cc.

The data on rabbit 15 show this drop in clearance to a greater extent. The first three periods were taken on the day before the experiment with the polysaccharide to ascertain normal behavior in the presence of the tumor. The following morning the animal was given 90 micrograms/kilogram body weight of the polysaccharide preparation. The body temperature reached a maximum of 41.5° 85 minutes before the start of period 4. It had been planned to start sampling 3 hours earlier, but adequate blood could not be obtained because of the state of the animal. The collapse progressed slowly, and the animal died some 8 hours later. Again the slight rise in blood uric acid could be explained by the failure of renal excretion or rather diminution of excretion to less than 20 percent of normal. The blood plasma creatinine was the highest encountered after administration of a standard dose, again reflecting the failure of excretion. The percentage rise in the proteose wave, while considerable as compared with the two immediately preceding periods, was within normal variations. Necropsy showed extensive hemorrhage in two large tumor metastases on the spermatic cord and on six retroperitoneal lymph nodes with blood-stained ascitic fluid. There were several metastases to the liver also, but here, as in none of the other experiments, they were grossly hemorrhagic.

The details on the experiment with rabbit 25 are given to contrast with the foregoing. This animal survived the dose of 30 micrograms/kilogram body weight, had much less tumor tissue, and no hemorrhagic areas attributable to the pyrogen. While the maximum temperature was less than that for rabbit 15 by only 0.6°, it never went into collapse, and showed a constant blood-uric-acid level in spite of the decrease in the hematocrit due to the extensive blood sampling.

Similar data for rabbit No. 20 are also shown as an example of those rabbits mentioned, to which a smaller dosage was given. At necropsy, this animal showed little tumor and no hemorrhage attributable to the polysaccharide. In general, this finding was true of all the animals given 5 micrograms/kilogram of body weight.

The main purpose of the macroscopic examinations was to determine (1) the approximate extent of the tumor involvement, (2) the relative degree of spontaneous necrosis, and (3) the hemorrhagic and necrotizing effect that might be ascribed to the recent treatment with the polysaccharide. We wished also to be sure that no spontaneous disease was present that might seriously affect the serologic determinations. Incidental to these examinations five of the rabbits were found to have small, localized, hepatic lesions, in which microscopic evidence of coccidiosis was found, and four animals showed a renal lesion, which consisted of extreme vacuolation of the cells of convoluted tubules affecting very small areas of the cortex. Neither the hepatic nor the renal lesions, however, were ever extensive, and it seems justified to consider that they produced no significant effect, for they were never directly associated with any change in temperature, blood chemistry, or urinary excretion.

Conditions associated with the polysaccharide treatment were congestion of the spleen, often to an extreme degree, in those animals that gave a severe reaction with considerable rise in temperature. This change in the spleen was often accompanied by cloudy swelling in the liver and kidney. Because of the variable degree of necrosis and hemorrhage, which usually develop spontaneously in the Brown-Pearce tumor, it was impossible to decide upon any particular degree of these conditions in the untreated control animals as a standard for comparison. It was not possible, therefore, to make a reliable comparative estimate as to how much increase in necrosis and hemorrhage might have occurred in the tumors of treated animals. An impression was gained, however, that the treated animals as a group appeared to show a more extensive necrosis that microscopically sometimes showed considerable destruction of peripheral as well as central areas. The hemorrhagic reaction likewise appeared more severe and often involved an entire tumor nodule instead of the scattered focal hemorrhagic areas which were often seen in the tumors of untreated rabbits. Several animals also developed a blood-stained ascites.

726906—47——3

Evidence of what appeared to be spontaneous regression of the Brown-Pearce tumor was observed microscopically in six experimental and in two control animals. The histologic picture was well described by Appel, Saphir, and Strauss (*24*). These authors first planted the tumor in the testis, then produced regression by a second implantation into the skin. Characteristic features were—

> degeneration and necrosis in the central portions of the tumors, while the marginal zones survived for a considerable time; infiltration by many lymphocytes and by a few polymorphonuclear and endothelial leukocytes; formation of foreign body giant cells; formation of tumor giant cells, with calcification of nuclei and deposition of calcium throughout the regressing transplants; failure of the new host to supply a vascular stroma (*24, p. 325*).

In our material, tumor giant cells were infrequent, and peripheral lymphocytic reaction was not an outstanding feature. Masses of large cells with finely vacuolated cytoplasm were often found at the margin of the regressing tumor in the testis. These resembled lipoid storage cells and probably resulted from the ingestion by histiocytes of substances accumulating during the destruction of the invaded normal tissue and from the tumor necrosis. Stains for iron showed a considerable accumulation of iron-positive pigment in this marginal zone. Phagocytic cells filled with dark-brown-to-black pigment were likewise found adjacent to regressing metastatic tumors of the eye. Very large foreign body giant cells with central calcification were also frequent.

This microscopic picture was remarkably uniform and distinctive in both the six experimental and the two control animals. Whatever the factors were that brought about these regressions independent of the polysaccharide administration, they were not recognized. All our animals were handled in the same way until the time of the polysaccharide treatment.

In our experience rabbits bearing the Brown-Pearce tumor have shown wide variation in the extent of growth and metastases during a given period. Spontaneous necrosis and hemorrhage have also varied in degree, and regressions have been frequent and unpredictable. For these reasons, the Brown-Pearce tumor proved unsatisfactory for an estimation of the necrotizing and hemorrhagic effect of the polysaccharide. On the other hand, where an effect on the blood proteose and temperature in a tumor-bearing animal was to be determined, for which a large laboratory animal was desirable, the Brown-Pearce tumor was perhaps the most satisfactory available. The presence of viable tumor cells and an approximation of the relative amounts of tumor tissue could be determined.

DISCUSSION

From the foregoing results, it is apparent that in those animals given the maximum tolerated dose for all animals, there was no clear-cut increase in the blood proteose concentration measured in this way. This does not exclude the probability that in other species or tumor types there might not be a pronounced increase of this moiety following treatment with the polysaccharide. Had there been widespread and severe damage to the tumor, the resulting tissue destruction might have given rise to an increased proteose content; but under the circumstances where the sensitivity to the polysaccharide, of the animal bearing the tumor, was so much of a limiting factor, no such increase was observed.

Brues and Shear (*5*) in discussing their four patients with inoperable cancer given the polysaccharide mention finding—

> marked and rapid increase in uric acid and nonprotein nitrogen in the blood of two patients * * * * * * * The two patients showing rapid increases in blood nitrogen had urinary-tract complications. In case 1, the period of anuria, which was related to the hypotension, may have been responsible for these increases (*5, p. 205*).

That there was little increase in blood uric acid in the animals under study, as reported in this paper, is perhaps likewise attributable to the lack of extensive tissue or cellular destruction. Insofar as there were any increases, they could be accounted for on the basis of decrease in renal excretion, without resort to the other logical explanation for such increases such as altered metabolism of nucleoprotein or of purines. The data reported herein tend to bear out the thought of the previous workers, that urinary-tract complications, especially anuria or deceased glomerular filtration, may be reflected as a factor in the increased blood uric acid. Renal function in rabbits and man is far from comparable, so that inferential transfer of data from one to the other is not completely possible. Also, perhaps it should be stressed once more that the polysaccharide had little effect on this tumor in the rabbit, whereas in other animals it produces much more of a reaction. Likewise, the dose level found herein to kill no rabbits is much below the minimum effective dose for mouse sarcoma, so that insofar as such a comparison is valid, little damage to this tumor of the rabbit might have been expected on the basis of this limitation.

SUMMARY

Adult male rabbits bearing the Brown-Pearce carcinoma were treated with a single dose of the polysaccharide from *Serratia marcescens* by intraperitoneal injection.

The dose of 5 micrograms/kilogram of body weight of the polysaccharide was found to be that which all animals tolerated.

This dose produced very little hemorrhage in the tumor tissue over and above what might occur spontaneously; with larger doses, a state simulating peripheral vascular collapse ensued in the presence of extensive tumor. With doses about 10 times this amount, there were hemorrhages within the tumors, ascites, decrease in renal clearance, collapse, and death.

Blood plasma proteose and uric acid were found to be little affected by the tolerated dose of 5 micrograms/kilogram body weight, even though this dose produced some systemic response and a small elevation of rectal temperature.

REFERENCES

(1) Winzler, R. J., and Burk, D.: Blood proteose and cancer. J. Nat. Cancer Inst. 5: 417–428 (1944).

(2) Westfall, B. B., Thompson, J. W., and Burk, D.: Polarographic response of deproteinated serum of individual rabbits before and after implantation with the Brown-Pearce carcinoma. J. Nat. Cancer Inst. 5: 407–414 (1945).

(3) Merten, R., and Bernot, J.: Ein Beitrag zur Isolierung und Verteilung der polarographisch wirksamen Gruppen in Serum unter besonderer Berücksichtigung von Krebssera. Ztschr. f. klin. Med. 139: 145–156 (1941).

(4) Hartwell, J. L., Shear, M. J., and Adams, J. R. Jr.: Chemical treatment of tumors. VII. Nature of the hemorrhage-producing fraction from *Serratia marcescens* (*Bacillus prodigiosus*) culture filtrate. J. Nat. Cancer Inst. 4: 107–122 (1943).

(5) Brues, A. M., and Shear, M. J.: Chemical treatment of tumors. X. Reactions of four patients with advanced malignant tumors to injection of a polysaccharide from *Serratia marcescens* culture filtrate. J. Nat. Cancer Inst. 5: 195–208 (1944).

(6) Shwartzman, G., and Michailowsky, N.: Phenomenon of local skin activity to bacterial filtrates in the treatment of mouse sarcoma 180. Proc. Soc. Exper. Biol. & Med. 29: 737–741 (1932).

(7) Franke, F. E.: Attempts to produce anaphylaxis in guinea pigs with a polysaccharide from *Serratia marcescens* (*Bacillus prodigiosus*). J. Nat. Cancer Inst. 5: 173–177 (1944).

(8) Franke, F. E., and Richert, D.: Effects of sublethal doses of a polysaccharide from *Serratia marcescens* (*Bacillus prodigiosus*) on the electrocardiogram, blood ascorbic acid, and nonprotein nitrogen of the dog. J. Nat. Cancer Inst. 5: 179–183 (1944).

(9) Franke, F. E.: Action of toxic doses of polysaccharide from *Serratia marcescens* (*Bacillus prodigiosus*) on the dog and guinea pig. J. Nat. Cancer Inst. 5: 185–193 (1944).

(10) Brown, H.: The determination of uric acid in human blood. J. Biol. Chem. 158: 601–608 (1945).

(11) Bonsnes, R. W., and Taussky, H. H.: On the colorimetric determination of creatinine by the Jaffé reaction. J. Biol. Chem. 158: 581–591 (1945).

(12) Fujita, A., and Iwatake, D.: Bestimmung der echten Blutzuckers ohne Hefe. Biochem. Ztschr. 242: 43–60 (1931).

(13) Cowgill, G. R., and Drabkin, D. L.: Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. Am. J. Physiol. 81: 44–61 (1927).

(14) Taylor, F. B., Drury, D. R., and Addis, T.: Regulation of renal activity. Am. J. Physiol. 65: 55–61 (1923).

(15) Hall, V. E., and MacGregor, W. W.: Relation of kidney weight to body weight in the cat. Anat. Rec. 69: 319–331 (1937).

(16) Kaplan, B. I., and Smith, H. W.: Excretion of creatinine, xylose and urea in the normal rabbit. Am. J. Physiol. 113: 354–360 (1935).

(17) Richards, A. N., and Plant, O. H.: The action of minute doses of adrenalin and pituitrin on the kidney. Am. J. Physiol. 59: 191–202 (1922).

(18) Stewart, G. N.: Possible relations of the weight of the lungs and other organs to body weight and surface area (in dogs). Am. J. Physiol. 58: 45–52 (1921).

(19) Brody, S.: Bioenergetics and growth. Ed. 1, pp. 352–403. Reinhold Pub. Co., New York (1945).

(20) Tui, C., and Schrift, M. H.: A tentative test for pyrogen in infusion fluid. Proc. Soc. Exper. Biol. & Med. 49: 320–323 (1942).

(21) Probey, T. F., and Pittman, M.: The pyrogenicity of bacterial contaminants found in biologic products. J. Bact. 50: 397–411 (1943).

(22) Beck, L. V.: The Shear tumor necrotizing bacterial polysaccharide as a pyrogen. Fed. Proc. Am. Physiol. Soc. 5: 6–7 (1946).

(23) Beck, L. V., and Fischer, M.: Effect on rectal temperatures in normal rabbits of the Serratia marcescens tumor-necrotizing polysaccharide of Shear. Cancer Research 6: 410–420 (1946).

(24) Appel, M. Saphir, O., and Strauss, A. R.: Morphologic alterations in the regressing Brown-Pearce tumor and their relation to its changes due to irridation. Arch. Path. 31: 317—325 (1941).

OBSERVATIONS ON THE IMMEDIATE RESPONSE OF PARAMECIUM TO MIXTURES OF AEROSOL-ACETONE AND AEROSOL-ACETONE-METHYLCHOLANTHRENE [1]

By GEORGE E. DANIEL, *special research fellow, National Cancer Institute, National Institute of Health, United States Public Health Service* [2]

INTRODUCTION

The literature contains a vast amount of fact and speculation concerning the intermediate and terminal phases of the action of carcinogens. Little or nothing is known about the initial site or mode of attack of chemical or radiant carcinogens on the cell. The terminal picture is probably the result of the response of various parts of the cell to a complex series of events set in motion by the initial contact of cell and carcinogen.

At the lowest level of biologic organization, a desirable experimental complex contains as one factor a cell which can be handled as an individual entity. Mottram (*1*) and Spencer and Melroy (*2*) have, among others, used *Paramecium* in experiments with carcinogens. In these experiments, the free-living cell was exposed to the carcinogenic agent several weeks to several years. These authors point out the likelihood of studies on such animals contributing information helpful in understanding the cancer process.

The free-living protozoa are being used as experimental cells in a continuing study, being carried on in this Institute concerned with the site and mode of action of certain of the chemical carcinogens. When the experiments reported in the present paper were first started, the aim was to see if any immediate effect of methylcholanthrene could be detected. Such an effect was not observed, but reactions due to the vehicle in which the carcinogen was presented to the cell were encountered. These reactions seem of sufficient importance to justify reporting them.

The data presented in this report deal with the immediate reaction of *Paramecium caudatum* (a free-living, ciliated protozoan) to a solution of the dioctyl ester of sodium sulfosuccinate (Aerosol O. T.) containing 20-methylcholanthrene [3] to plain aerosol solution in water and to mixtures of aerosol solution and acetone and ether. Attention has been directed to cell reactions which occur in a relatively short time. This has the advantage of eliminating to a large degree complicating factors such as the ingestion of food, intrinsic changes in the test solutions with time, or the accumulation of waste products from the cell.

MATERIALS AND METHODS

Mass cultures of *Paramecium caudatum* 4 to 5 days old served as sources of experimental material. Cultures were grown in 2-ounce specimen bottles each containing balanced saline solution, four rice grains, and a mixed bacterial flora. The bottles were closed with rubber stoppers. Routine transfers were made at 10- or 12-day intervals.

Using aerosol as a wetting agent, Lorenz, Shimkin, and Stewart (*3*) developed a method for the colloidal dispersion of several carcinogenic hydrocarbons and hormones. The procedures used in the present study were based on the studies of these authors.

Two methods of making colloidal suspensions of MC were used. In the first, acetone was used as a solvent for the hydrocarbon. Five cubic centimeters of acetone containing the desired amount of MC was added drop by drop from a funnel to 250 ml. of boiling aerosol solution in a 1,000-ml. flask. The inside of the funnel and the outside of the stem were rinsed with an additional 4 ml. of acetone. Boiling was continued 3 to 5 minutes beyond the point where no odor of acetone was present. During the boiling process, 50 to 60 ml. of fluid was lost. After cooling, the volume was restored to 250 ml. with distilled water, and any further dilution desired was made with plain aerosol solution. Ultraviolet absorption spectra, made with the Beckman photoelectric spectrophotometer, showed no evidence of free acetone in solutions so prepared. Throughout these experiments, 0.005-percent aerosol solutions were used. In order to have proper control solutions an identical procedure, employing MC-free acetone was used for making aerosol-acetone and water-acetone mixtures.

The second method of preparing suspensions involved the use of ether as the MC solvent and the removal of the solvent under vacuum at room temperature. In this method, 9 ml. of purified ether containing the MC was added dropwise, with constant shaking, to 250 ml. of 0.005 percent aerosol. Suction flasks containing solutions of aerosol-ether or aerosol-ether-MC were attached by a common manifold to the house vacuum. Examination of samples in a Westinghouse mass spectrometer showed that after 96 hours under vacuum all ether was removed from the solutions.

When paramecia are placed in a 0.005-percent aerosol solution in distilled water, osmotic swelling leading to the death of the cell occurs. In many instances, the cell wall is ruptured before motion of the cell ceases; in other cases, cessation of motion precedes rupture. In this study, the time from first immersion until cessation of visible motion, or rupture of the cell, whichever occurred first, was measured. Observations were made

[1] Received for publication October 29, 1946.
[2] The author is indebted to Mrs. Dorothy Peterson, Physics Section, Industrial Hygiene Research Laboratory, of the National Institute of Health, for making the ultraviolet absorption determinations.
[3] Hereafter referred to simply as MC.

with a wide-field binocular microscope at 30 × magnification.

Spot plates of clear pyrex glass, each containing three rows of three depressions, were used for observation dishes. Approximately 0.9 ml. of solution was put in each depression. A single paramecium, selected at random from a group of large, actively moving organisms, was picked up and put in the first depression of one row. In order to minimize the amount of material carried over from the mass culture, the organism was transferred in rapid succession through the first two depressions in the row and left in the third until death.

A stop watch was used to determine the killing time of each individual paramecium. Time was recorded to the nearest second. In every instance, part of the depressions on each plate contained experimental and part control solutions. Each curve in the various figures is based on from 3 to 5 experiments, with 10–15 paramecia in each, using different cultures and experimental solutions prepared at different times.

Dependent on the cleanliness of the dishes and purity of the water, *Paramecium* may live in distilled water for many hours or only a few minutes. Since under the conditions of these experiments, the killing time in water was not so uniform or so short as in aerosol solution, a period of 30 minutes was arbitrarily chosen as the duration of the experiments reported in table 1.

TABLE 1.—*Paramecia alive or dead after 30 minutes' exposure to distilled water and to distilled water to which acetone was added and subsequently removed* [1]

Experiment No.	Exposure of paramecia to—			
	Distilled water		Distilled water with acetone removed	
	Alive	Dead	Alive	Dead
	Number	*Number*	*Number*	*Number*
1	1	8	2	7
2	7	2	8	1
3	8	1	4	5
4	4	5	8	1

[1] The acetone was removed before the paramecia were exposed.

RESULTS

Figure 1 shows the results of comparisons between solutions of plain aerosol (aerosol to which no acetone or other MC solvent has been added and removed), aerosol-acetone, and 0.986 microgram MC per milliliter in aerosol (the MC added as acetone solution as previously described). These curves fall into two groups: (1) Plain aerosol; and (2) MC-aerosol-acetone and aerosol-acetone.

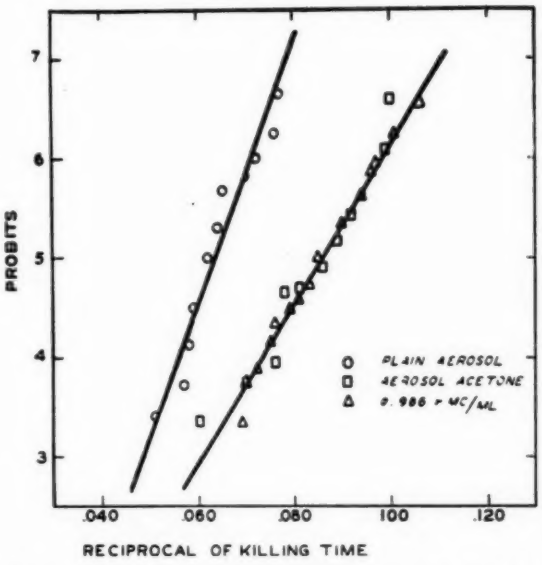

FIGURE 1.—Comparison between the reciprocal of the killing time of paramecia in aerosol, aerosol-acetone, and aerosol containing MC.

There is a significant difference between the groups, but aerosol-acetone and MC give identical curves. These results show that some substance

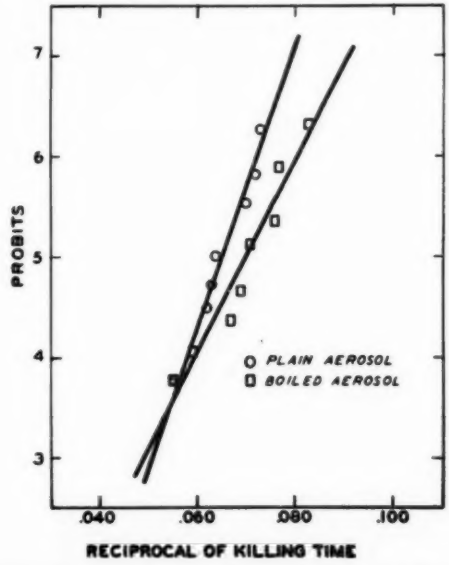

FIGURE 2.—Comparison of the reciprocal of killing time of paramecia in boiled and plain unboiled aerosol of the same concentration.

other than MC in the hydrocarbon suspension is responsible for the effects observed.

The data were analyzed according to procedures outlined by Bliss (*4*). For the purpose of statistical analysis [3] and for the preparation of charts, the killing times were converted to their reciprocals and percentages to probits (cf. Bliss (*4*, *5*)).

The plain aerosol solution was not boiled during preparation while the MC colloid was vigorously heated. A comparison of boiled aerosol with unboiled (fig. 2) shows no difference in the effect of the two solutions.

The results of a comparison between distilled water and distilled water-acetone (table 1) indicate that acetone, added to and subsequently removed from distilled water, does not increase the toxicity of the water. It seems unlikely, therefore, that acetone as such is responsible for the effects shown in other figures of this paper.

Figure 3 shows a comparison of unboiled aerosol, aerosol-ether, 0.986 micrograms MC per milliliter, and 1.97 micrograms MC per milliliter. There is no significant difference between the curves.

Ultraviolet absorption spectra, as measured by the Beckman photoelectric spectrophotometer, gave identical absorption curves for aerosol-acetone-water and for aerosol-water.

DISCUSSION

While nothing is known concerning the mechanism of the action, the data indicate that the mixture of aerosol and acetone is of such nature as to affect *Paramecium* adversely. Although the ultraviolet spectra and the mass spectrometer data fail to indicate the presence of any acetone or new compound in the solution, the sensitivity of both methods of measurement is limited, and the amount of material to which *Paramecium* is sensitive may well be below the limits of the physical methods used. With this possibility in mind, it may be suggested that a toxic compound is formed or that a trace of one of the components of the solution so changes the cell that the other component is able to exert an influence leading to rapid death.

In view of the results obtained with *Paramecium*, it appears that in any experiments designed to study the action of methylcholanthrene as such, the possible effects of the solutions or mixtures in which the methylcholanthrene is presented to the experimental material must be carefully considered and controlled. Not only the separate components of the solution but also the complexes formed between materials ordinarily considered inert must be considered.

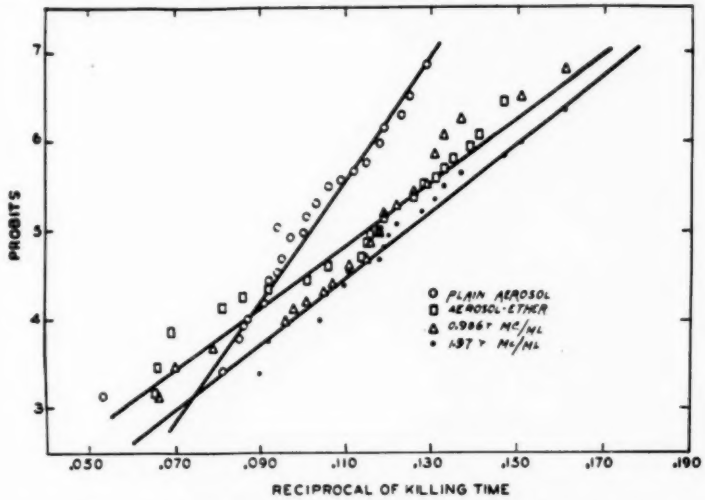

FIGURE 3.—Comparison of the reciprocal of killing time of paramecia in plain aerosol, aerosol-ether, and two concentrations of MC.

SUMMARY AND CONCLUSIONS

An aerosol-acetone-water mixture is lethal for *Paramecium caudatum* in a significantly shorter time than is an aerosol-water or an acetone-water mixture, the concentration of the several substances being equal in different mixtures.

Ether-water-aerosol mixtures are no more toxic than water-aerosol mixtures.

The addition of 20-methylcholanthrene to acetone-water-aerosol or ether-water-aerosol mixtures

[3] Dr. W. Ray Bryan, of the National Cancer Institute, suggested the statistical procedures used in this report.

does not significantly change the killing time from that found in the same mixtures without the methylcholanthrene.

If an acetone-water-aerosol mixture is present in an experimental environment, its effect upon cells must be considered in all work designed to study the mode of action of methylcholanthrene as such.

REFERENCES

(1) Mottram, J. C.: Abnormal paramecia produced by blastogenic agents and their bearing on the cancer problem. Cancer Research 1: 313–323 (1941).

(2) Spencer, R. R., and Melroy, M. B.: Effect of carcinogens on free-living organisms. Survival value of methylcholanthrene-adapted paramecium. J. Nat. Cancer Inst. 1: 343–348 (1940).

(3) Lorenz, E., Shimkin, M. B., and Stewart, H. L.: Preparation of dispersions of carcinogenic hydrocarbons and hormones with the aid of dioctyl ester of sodium sulfosuccinate (Aerosol O. T.). J. Nat. Cancer Inst. 1: 355–360 (1940).

(4) Bliss, C. I.: The calculation of the time mortality curve. Ann. Appl. Biol. 24: 815–852 (1937).

(5) ———: The calculation of the dosage mortality curve. Ann. Appl. Biol. 22: 134–167 (1935).

AVENUES OF APPROACH TO THE GASTRIC-CANCER PROBLEM[1]

By MORRIS K. BARRETT, M. D., *senior biologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

Neoplastic disease is one of the greatest scientific problems facing us today. It is a challenge to the clinician and the investigator alike. Second only to heart disease as a cause of death, it goes on year after year piling up a casualty list far in excess of that due to war. Plans for a major attack upon the problem are being laid in many quarters. It is not universally recognized, however, that of the many categories into which the cancer problem may be divided, gastric cancer commands especial attention. This is true because of clinical and statistical considerations.

Let us examine for a moment the statistical characteristics of gastric cancer. Various authorities estimate that from 26,000 to 40,000 people in the United States die of gastric cancer each year. The only other clinical forms approaching this mortality rate are cancer of the intestine, which causes approximately 20,000 deaths per year, and cancer of the uterus and breast, each of which cause about 16,000 deaths per year. With this high incidence, gastric carcinoma has the lowest survival rate of any frequently encountered form of the disease. Pack and Livingston stated that—

> if every patient with gastric carcinoma entered the best surgical clinic in the world, 95 percent would remain uncured and approximately 90 percent would be dead of the disease in 18 months.

Walters, Gray, and Priestley reported a 5-year survival rate of 6.2 percent of 11,000 cases seen at the Mayo Clinic during a period of 32 years. Others have reported survival rates in selected groups of cases which were higher, but all are agreed that the prognosis in gastric cancer is poor indeed.

Most gastric-cancer patients are beyond the stage in which a cure may be hoped for before they are admitted to a hospital. Some improvement in survival rates among resectable cases has been made since the time of Billroth, but over-all survival rates are still discouragingly small. In the most favorable group of cases, a 5-year survival rate of 50 percent has been attained, yet the over-all survival rate reported from most clinics is scarcely one-tenth of that. The great difficulty is that not many cases are in the most favorable group at the time when they are first seen in the clinic. Instead, the average hospital report shows that, of all patients admitted with cancer of the stomach, about half are operable. Of the operable group, about half are resectable; and it is from this fourth of all cases that a "most favorable" group must be selected.

Obviously then, if numbers mean anything, the crux of the problem lies in earlier diagnosis. Barring some at present unknown radical innovation in cancer therapy, we shall not materially improve the situation until we have learned some method of diagnosing cancer of the stomach earlier in the course of the disease. Methods of prevention may be even more remote. Hurst in 1939 expressed the view that—

> the onset of cancer of the stomach is so insidious and diagnosis so difficult that, even with modern methods, it is doubtful that the most skillful surgeon will ever increase the 5-year survival rate much beyond the present maximum of 5 percent of all cases and the only hope lies in prophylaxis.

How may so great a problem be attacked? A re-examination of the meager facts available may suggest feasible methods of attack. Someone may see the old facts in a new light, or perhaps new recruits may be obtained for the altogether too small group of workers now engaged in the field. In view of the enormity of the problem, this seems worth while.

In preparation of the digest which follows, the literature was scoured for fact and for hint. Over 500 articles and books were read, and an equal number was scanned. Obviously, a complete set of references given in the usual manner would be too ponderous to serve any useful purpose. A large bibliography arranged in divisions similar to those used in the text is appended. A selection was made of outstanding or key articles and those needed to establish the facts set forth. Many case reports and minor articles covering the same material as the references given were omitted. Undoubtedly some significant papers were overlooked unknowingly. In some cases, foreign publications were not available.

A single statement of the consensus of several authors is made where it seems desirable. In other cases direct quotations giving authorship are used for outstanding contributions, review articles, or where it is necessary to afford the reader a proper entry into the literature. It is not meant to assign credit for priority by giving an author's name or to withhold credit by omitting it. The form used was dictated by the need for condensation, and it is regretted that in many cases authorship is indicated only by the entries in the bibliography.

This is not a field that abounds in proved facts; reasonable assumptions are far more common. In interpreting and evaluating the material, it is considered advantageous to adopt an attitude of mind neither too rigorously scientific nor yet too gullible. It seems desirable in attacking such a complex and unknown problem to make free use of working

[1] Received for publication November 20, 1946.

726906—47——4

hypotheses and to allow the imagination considerable freedom. Perhaps we shall advance more rapidly if we occasionally venture onto unfirm ground.

In addition to the introductory remarks and general considerations, there are 12 major sections into which the associations and relationships of gastric cancer are divided in this paper. At the end of each section, an attempt is made to point out the significance of the facts reviewed. Where possible, the applications of the knowledge to cancer control, clinical experimentation, and animal experimentation are suggested.

GENERAL CONSIDERATIONS

As may be said for most types of cancer, the etiology of gastric cancer in man is not known. There are, however, certain clinical associations of the disease which are suggestive, and these are discussed herein.

Gastric cancer is a disease of later life, the average age of patients being about 55 years. However, McNeer collected 501 cases occurring earlier than the thirty-first year, of which the youngest was less than 5 years old.

There is a preponderance of cases in the male. All reports agree qualitatively on this, but we meet here one of the problems for solution by statisticians and epidemiologists. Why is it that statistics collected by public agencies show a sex ratio of about 1.6 males to 1 female, while so many reports from clinics indicate a ratio of as much as 3.7 to 1?

Collins, Gover, and Dorn reported a higher mortality rate for cancer of the stomach and duodenum in the Northern States than in the Southern. The same trend is indicated in a report by States published this year. This might be taken as an indication of a climatic or geographic variation in the incidence of the disease. On the other hand, an inspection of the latter report reveals that the same group of Southern States that reports a percentage of gastric cancer well below the United States median also reports a ratio of the number of physicians to the population which is well below the average. This may mean that the true explanation lies in differences in availability of diagnostic facilities rather than in climate. It also seems to be one of the many indications of the need for better statistics collected through greater and more uniform diagnostic and statistical facilities.

From time to time there have appeared reports of racial differences in the incidence of gastric cancer. Most of these have not been well authenticated and have been open to criticism on the basis of nonuniformity of diagnosis. The oft-quoted studies of necropsy material by Bonné indicated a difference between the Chinese and Malays in Java. There were but 5 gastric cancers in Chinese and 1 in Malays found in 2,015 and 3,885 necropsies, respectively. The statistical significance of this finding is questionable. Our knowledge of the different susceptibilities of various strains of inbred mice might lead us to expect that racial differences in man could exist (though races and strains are not strictly comparable); but if such is the case, it is unknown at present.

HEREDITY

If one speaks of genetics in an exact sense, it may almost be said that nothing is known of the genetics of gastric cancer in man and very little more is known with regard to animals. There are, however, some indications that heredity has the important influence that one would expect.

Geneticists have said that, in the absence of proof, it should be assumed that cancer in man is genetically controlled in some manner, and further that the genetic influence is probably organ specific. This is presumed because of cautious inferences from animal experimentation and certain observations on the disease in man, some of which are mentioned later. This should apply to gastric cancer as well as to any other. How important this effect is in the over-all situation and how it operates are not at present known.

Some statistics have been and are being collected. They are not yet adequate for analysis, but one may guardedly accept certain trends as a guide for future investigations.

As in other forms of cancer, there are exceptional families known for their high concentration of gastric cancer. The family of Napoleon is perhaps the most famous of these. Napoleon, his father, and grandfather, all 3 sisters, and 1 of 4 brothers are said to have died of gastric cancer. In 3 of them, the diagnosis was made by inspection at necropsy; the other 4 were "suspected." Oddly, no mention in this regard of further descendants of the family has been encountered. Then there is the family "G" originally reported by Warthin. At last report 174 members of this family were 25 years of age or older. There had been 43 primary carcinomas in 41 individuals, an incidence of 24 percent. Of these, 26 carcinomas were gastrointestinal, and 15 were in the endometrium. Such family histories make very interesting reading, but of course they are not scientific evidence. Blank called attention to the fact that, considered only as a matter of probability, in a disease as common as cancer 1 family in 10 will have 3 or more cases when families of 10 or more members are considered.

Von Verschuer and Kober investigated the occurrence of cancer in 188 pairs of twins. They

concluded that there was evidence for a hereditary influence on localization of the tumor but none for general predisposition. Further, with respect to gastric cancer this factor for localization was evident in the data for identical twins as compared with fraternal twins.

Macklin made a study of tumors in monozygotic and dizygotic twins and reached the following conclusions: In monozygous twins, as compared with dizygous, pairs in which both members develop a tumor are more frequent, the tumor is of the same type and in the same organ more frequently, and the age of onset is more nearly the same. Since monozygous twins have identical hereditary potentialities and dizygous twins do not, these three facts support the belief that heredity plays an important role in tumor production, site of occurrence, and age of onset.

Various observers have reported that a study of family histories indicates a familial connection between gastric cancer, pernicious anemia, and achlorhydria. It is suggested that in certain families there is some genetically controlled defect of the gastric mucosa. In one member of the family the defect, plus unknown influences, may eventuate in pernicious anemia; in another member the defect may reach final expression in a gastric cancer. The statistics are only suggestive, however, and not conclusive. It is interesting that the earliest occurrence of achlorhydria found in the literature was in a 10-year-old girl, who was the daughter of a woman who had pernicious anemia.

With respect to the laboratory animal, while much has been learned of the genetics of strains having other tumors, there is only one reported strain of mice said to develop spontaneous gastric adenocarcinoma. That is the NHO strain recently developed by Strong. Further reports on this strain are awaited. At this time, the principal points of interest are that the strain develops a considerable percentage of spontaneous lesions of the stomach and that Strong believes that the high incidence of the tumor in this strain was developed by the induction of a genetic mutation. Engelbreth-Holm, in a recent lecture, said that in his laboratory there is a strain of mice that is beginning to have some spontaneous gastric adenocarcinomas. One other strain of mice should be mentioned at this point. Andervont and Stewart described an adenomatous (but nonmalignant) lesion that regularly occurs in the stomach of Strong's strain I mice. The fact that this anomaly of the glandular mucosa occurs in a high percentage of the mice of this highly inbred line, together with the results of the hybridization studies of Andervont, indicates a genetic basis for the lesion.

The implications of all this for research from a genetic standpoint, we leave to the geneticists. Strong and his associates are carrying out a program which may be expected to yield valuable data. The production of other susceptible strains of mice and rats to be used in experiments with some of the more promising methods mentioned elsewhere would be of the greatest value. In the clinical field, we need more and better data. Present methods of collecting data in human cases are not adequate. The conference on parental influence in relation to the incidence of human cancer, at Bar Harbor, pointed out ways in which better data could be accumulated. However, the lack of large accumulations of dependable data is not a problem in the genetic field alone. The lack is seen in all statistical phases of the cancer problem and is discussed at greater length later.

DIET AND VITAMINS

The accumulation of evidence that gastric cancer is very common in man and very rare in animals made the idea that the cause lay in something peculiar to the diet of man very attractive. However obvious the connection may seem, the proof of a causal relationship has not been forthcoming. Grossman and Ivy in a recent commentary said "our present knowledge of the influence of diet on carcinogenesis poses more questions than it answers."

The best-known studies of the relation of diet to gastric cancer in man are those of Lintott and Herbert and Bruske. They compared a large group in Holland with a similar group in England. The incidence of gastric cancer in Holland was about twice that in England. The diet of the Hollanders contained a larger amount of bread, cheese, and vegetables, and a smaller amount of meat. Furthermore, in Holland, the food was taken at a higher temperature, and the consumption of spices, spirits, and tobacco was higher. Attempts by careful history taking to discover some significant characteristic of the diet of persons with gastric cancer in the United States have been unsuccessful thus far.

Other references to this subject are found in the bibliography, but no convincing evidence of a dietary factor for gastric cancer in man was encountered in this review.

The studies of the metabolism of cancer patients which is in progress at Memorial Hospital has revealed several abnormalities, notably vitamin A deficiency and hypoproteinemia. Some new light on nutrition and gastric cancer may come from these studies, but it is too early to evaluate them for our purpose. The same may be said of the study of patients with atrophic gastritis that is being made by Shapiro and co-workers. They be-

lieve that some nutritional deficiency is involved in the etiology of gastritis and reported some gastroscopic evidence of healing after choline treatment of patients having gastritis.

The most striking feature of the literature pertaining to the production of gastric cancer in experimental animals by dietary means is the lack of agreement among authors. Most reports of a positive result with a dietary regimen can be matched with a negative report. One of the principal difficulties arises from the fact that a large part of the work was done with the rat. The rat, as is well-known, has a very labile gastric mucosa. All sorts of procedures, including several nutritional deficiencies and simple starvation, have been reported to produce lesions in the rat's stomach. Brunschwig and Rasmussen discussed these reactions of the rat's stomach to a variety of unbalanced diets. Their results indicated that the reactions were nonspecific for any dietary factor tested. Apparently it is necessary to be very cautious in evaluating effects in this animal. In addition, a large portion of the stomach of the rat and of the mouse is lined by squamous epithelium. Most of the lesions reported were in the forestomach. It seems more logical to relate lesions of the squamous epithelium in the rumen of these animals to lesions of the mouth, pharynx, and esophagus in man than to compare them with adenocarcinoma of the stomach. This distinction is adopted in this review. The analysis of pertinent results is much simplified thereby.

There is no well-authenticated instance of the experimental production of an adenocarcinoma of the stomach by a dietary procedure. There are, however, certain suggestive findings in relation to the glandular epithelium after dietary experiments which should be recalled.

Pathologic lesions that may be related to cancer in ways not now known have been observed in the glandular mucosa of the stomach of rodents following the feeding of coal tar and tar products, fuel-oil distillate, tobacco tar, heated animal fats, cholesterol derivatives, a diet of white flour and salt mixture, and a diet of rice and olive oil. The lesions reported were adenomas, polyposis, ulceration with hyperplasia, and a few "adenocarcinomas." The last have not been accepted as undoubtedly malignant for reasons discussed by Klein and Palmer.

Roffo's frequent reports that "adenocarcinoma" is produced by feeding heated fats to rats require special attention for two reasons: (1) The agent employed has a popular appeal because of the presence of heated fat in the diet of man and the absence of similar substances from the diet of animals; and (2) (and perhaps in consequence of the first reason), there is evidence that some clinicians accept the implications of these reports and are trying to apply these ideas to patients. Indeed, the implications are too important to be taken other than seriously.

Klein and Palmer discussed Roffo's work and considered that the lesions did not meet accepted criteria for malignancy. The lesions were limited by the serosa of the stomach, no metastases were observed, it was not shown that they were irreversible in the absence of the stimulus, they were not transplanted, and there were similarities to less severe and nonmalignant lesions observed under other conditions.

At the National Cancer Institute, a group of workers has attempted to repeat Roffo's experiments with heated fats and obtained no carcinomas. 3, 5–Cholestadiene, which was said to be the active agent in heated fats, was tested directly, also with negative results. Beck, Kirby, and Peacock reviewed the work done along these lines and repeated some of it. They reported negative results after using several fat and cholesterol derivatives.

This is an important aspect of the gastric-cancer problem. Much work has been done, and we do not yet know why there should be so much discordance between the work of Roffo and that of British and North American workers. Even if it is accepted that the lesions reported by Roffo are nonmalignant, still his publications show a lesion of the glandular mucosa which is more severe and encountered more often than those lesions usually found in other laboratories where similar work is done. Clarification of this point would be desirable.

One explanation for the divergence of the results observed may be that the strain of rats used by Roffo are more prone to develop lesions in the glandular stomach than are the rats used by other workers. Or there may be some other unknown difference in the experimental conditions. We would attach considerable importance to the explanation of this disagreement.

At any rate, it would seem that until more evidence is available, we must consider that the case for heated fats and cholesterol derivatives is not proved.

The paper by Sugiura in Cancer Research for 1942 contains a very useful tabulation of reports dealing with this subject. In 1944 Rusch made a critical survey of extrinsic factors that influence carcinogenesis, which includes much that is related to dietary effects. Burk and Winzler in a general review of vitamins and cancer have a section on gastrointestinal cancer of considerable interest. We were not convinced by the data presented in the report of Iacobovici and colleagues that in the presence of gastric cancer the topographical distribution of vitamin C in the gastric mucosa is characteristic.

Experiments in which one of the common carcinogenic hydrocarbons was added to the diet or drinking water of mice or rats have not produced adenocarcinoma of the stomach thus far. Adenocarcinomas of the small intestine and squamous-cell carcinomas of the forestomach were observed, however. It seems from present results that the greater susceptibility of the squamous mucosa is one hindrance, and some sort of natural barrier, perhaps mucus, which protects the glandular mucosa may be another.

We have seen only two recent reports of dietary experiments in the dog which were related to the subject under discussion. Layne and Carey observed no change in the gastric mucosa of five dogs in which severe black tongue had been produced by deficient diet. The experiments were not carried beyond 102 days. Street and co-workers reported that in their experiments with the B vitamins in the dog there was a suggestion that deficiency in B_6 might depress gastric secretion.

No practical suggestion for cancer-control programs is obtained from this review of dietary experiments in cancer. The suggestion that people should boil meat instead of roasting or frying it is not based on sound evidence. It might be interesting, however, to collect data on those people that customarily leave frying fats in the pan through many reheatings. Tannenbaum's suggestions regarding persons who are overweight should be mentioned at this point. He reported that caloric restriction decreases the incidence of spontaneous or induced tumors in animals. From the statistics of insurance companies, he found that more policy holders who were overweight at the time that the policy was issued died of cancer than did those who were normal or underweight. This is an interesting observation, and the further collection of data is desirable.

As a guide for further clinical investigation, there is only the suggestion by Ivy that a study be made of special localities or groups in which the incidence of gastric cancer and dietary habits are markedly different from the usual.

For animal experimentation there are several suggestions. Perhaps the most valuable of these is to repeat some or all of the more encouraging dietary experiments in a strain of mice with a high incidence of spontaneous gastric cancer when such animals become available. The adenomatous gastric lesion of strain I mice might also be used as a substrate for the testing of the influence of various diets. An investigation of the strain of rats used by Roffo for special susceptibilities of the gastric mucosa would certainly be of great interest. Then there is the suggestion that the gastric atrophy which Thomsen observed in a dog after the administration of alcohol may have been due to a dietary deficiency. Against this is the fact that in man, both Gray and Schindler were unable to correlate the incidence or severity of the gastritis observed in chronic alcoholics with vitamin deficiencies. It has been suggested repeatedly that any of the methods, dietary or otherwise, that produce ulceration in the rat's stomach may also destroy the barriers by which the normal mucosa is protected. Methods for combining the action of a carcinogenic chemical such as methylcholanthrene, etc., with procedures which will maintain ulcerations of the glandular mucosa, i. e., nutritional deficiencies, are worthy of a trial. A good suggestion along those lines is that made by Ivy. He suggests using the rabbit, which when on a rough diet tends to heal experimentally induced ulcers with adenomatous overgrowth. In all such studies, the experiments should be carried on for long periods before negative conclusions are accepted.

It is evident that for diet alone a review such as this discloses enough problems and suggestions to keep investigators busy for some time.

HORMONES

No direct evidence that hormones are concerned in gastric cancer has been found. Certain inferential material should nevertheless be considered in a general review of the problem such as is attempted here.

The primary suggestion that hormonal influences might be of importance in gastric cancer comes from the fact that the disease occurs more often in men than in women. This apparently does not hold for young patients, for McNeer found that in 501 cases occurring prior to the fourth decade, the sex incidence was equal. Contributory observations are that males are more subject to gastric ulcer and that there are sex differences in the secretion of acid by the stomach, particularly with respect to the sharp rise in average gastric acidity that occurs in the male after puberty.

Next there are numerous observations that the endocrine system affects gastric physiology in various ways, which may or may not be directly related to cancer. This subject was reviewed recently by Abrahamson and Hinton. At the risk of some confusion, a few of the findings are recounted here.

It has often been reported that in women with peptic ulcer there is a remission of the ulcer symptoms during pregnancy. A high incidence of histamine-fast achlorhydria has also been reported in pregnant women. In the dog, a marked increase in volume and rate of acid secretion has been observed at parturition and during lactation. A recent report by Way of a study of seven pregnant women states that there is an inverse relation between the gastric acidity and the anterior pitui-

tarylike hormone content of the urine. Sandweiss, Saltzstein, and Farbman demonstrated an "anti-ulcer" substance, "anthelone," in the urine of pregnant women and normal men and women which had a prophylactic and therapeutic effect on the ulcers of Mann-Williamson dogs. The substance is associated with but not the same as anterior pituitarylike hormone. Favorable or encouraging results have been reported in clinical trials of estrogens in males with ulcer although there may be an increase in acid. The facts were not well-established, however. In dogs with Pavlov pouches, a depression of volume, free acid, and total acid has been observed after injection of commercial preparations of anterior pituitarylike hormone, but no change was produced by theelin. Antuitrin-S is said to inhibit the development of ulcer in Mann-Williamson dogs, and stilbestrol is reported to inhibit the formation of ulcer in dogs treated with cinchophen. All this seems to indicate that a study of the sex hormones and their close relatives will lead to a better understanding of their complex relationship with gastric physiology and thence to useful knowledge in gastric cancer.

Parenteral injection of pitressin (or barium chloride) was reported by Dodds et al. to produce a necrotic lesion in the stomach of a rabbit, but Atkinson and Ivy observed no effect in Pavlov pouch dogs after administering 1 cc. per day for 2 weeks. Perhaps at this point we should recall, without going into detail, that since the classic experiments of Cushing, many workers have shown that injury to the hypothalmus produces ulceration of the gastric mucosa. Loss of glandular tissue and lymphoid accumulations were observed in the stomachs of hypophysectomized dogs by Magruder. Sandweiss recently reported preliminary data indicating that after destruction of the hypophysis in dogs, urogastrone may disappear from the urine, and in some cases the urine extracts stimulate gastric secretion.

An increased incidence of achlorhydria in hyperthydroidism has been reported by several observers. Brown, Pendergrass, and Benedick reviewed this field in 1941. The literature is somewhat conflicting, though in general it indicates an inverse relationship between thyroid activity and stomach acidity both in man and the experimental animal. Degenerative changes in the gastric mucosa were reported in myxedema in man, and reversible changes were reported in experimental thyrotoxicosis in the dog.

Babkin and co-workers reported that inconclusive results indicated that the administration of parathormone (or activated ergosterol or intravenous calcium lactate) to the dog in doses which raised the blood calcium to 15 or higher would suppress the nervous phase of gastric secretion.

Tuerkischer and Wertheimer recently reported that in rats there is a marked depression of gastric secretion following total adrenalectomy. Medullectomy alone does not depress gastric secretion. The gastric function is restored by administration of adrenal cortex but not by sodium chloride or desoxycorticosterone.

The subject of the hormones of the gastrointestinal tract itself will not be covered completely, since Ivy recently reviewed this field. There are, however, four substances of a humoral nature, not all established as hormones, that are of great interest here. These are the secretory depressants enterogastrone, urogastrone, and the material extracted from achlorhydric gastric juices by Brunschwig, and the anti-ulcer factor, anthelone. The last was mentioned previously. The first three depress gastric secretion on parenteral administration, the fourth does not. There is another, and for our purposes perhaps even more important, effect of enterogastrone, urogastrone, and anthelone. It has been shown that after prolonged administration of these substances to Mann-Williamson dogs, the animals become resistant to ulcer formation after withdrawal of the treatment. Apparently some "immunity" is set up in the animal which alters the processes controlling tissue injury or healing and repair. Furthermore, in such "immunized" Mann-Williamson dogs there is a permanent alteration in the animals' response to an alcohol test meal. Their response is abnormal before treatment but returns to normal after treatment.

Friedman et al. reported that urogastrone is present in the urine of patients with gastric carcinoma or pernicious anemia, but relative amounts were not reported.

The exact relationships and chemical natures of enterogastrone, urogastrone, and anthelone are not known at present. Necheles recently pointed out the problems involved in the final elucidation of the nature of these substances, and Brunschwig discussed the possibility of a relationship between enterogastrone and the secretory depressant found in gastric juice.

In addition to the great importance of these substances from the standpoint of gastrointestinal physiology and peptic ulcer, they concern us here because it is easy to imagine that a hormonal imbalance of one or all of these factors might underlie the secretory disturbances or faulty tissue resistance that seem so important in gastric carcinogenesis.

An attempt to interpret the foregoing facts in their present sketchy state does not appear feasible. Until more evidence is available, it is better to consider each separate item with regard to its own possibilities.

The last four substances seem at present to offer the most promising field for future experimental work. The results obtained by the large group of investigators already at work in this field will be eagerly awaited. The influence of the hypophysis and gonads appears to be the next most promising suggestion. Most of this work has been done in the dog. Since it now appears that in the near future special strains of mice may be available for gastric investigations, it would be useful if some of the observations were extended to this species. Investigations of the status of gastric cancer patients in regard to the secretory depressant hormones (chalones) have already been started in various laboratories as indicated previously.

Ivy mentioned the possibility that there might be an interrelationship among enterogastrone, achlorhydria, gastric cancer, and chronic gastritis by reason of the pylorization or intestinalization of the gastric mucosa that may be present. Somewhat against this is the failure of several observers to confirm the depressant effect of jejunal implants reported by Andrus et al. This is a highly speculative matter, but the original suggestion, which was made prior to the latter observations, still contains interesting possibilities.

For cancer control, there is little to be extracted from the preceding material that is of practical use. It might be well, however, to recall when using or advocating the use of steroid hormones for the treatment of gastric ulcer that some of the patients so treated may already have a lesion which is cancerous or precancerous and that these hormones have been shown to be carcinogenic (for tissues other than genital), though none have as yet produced cancer of the stomach so far as is known. This may or may not be important, but it should be kept in mind.

BACTERIOLOGY

The earliest report of the experimental production of gastric cancer (Fibiger 1913) ascribed the lesion to a nematode worm infesting rats. The interpretation was later shown to be erroneous. In 1939 Bonné and Sandground reported a "near malignant" gastric "adenopapillomatosis" associated with a similar parasite. A bacterial or viral cause for stomach cancer has been sought by many but not found.

Saunders in 1931 isolated an anaerobic streptococcus from cancer of the breast, cervix, colon, and stomach and proposed a hypothetical etiologic relationship. This has not been established.

Extensive bacteriologic studies of a large number of both healthy and diseased stomachs have been reported in recent years. The most recent of these is that by Barber and Franklin. The findings are in general agreement and are as follows: The healthy stomach is usually sterile. Total acidity of about 40 degrees after an Ewald meal seems to be the limit above which positive cultures are seldom obtained. Pathogenic and nonpathogenic organisms, including a large number of common types are found in the presence of chronic gastritis, pernicious anemia, gastric cancer, and some ulcers. Their presence seems to be correlated with low acidity or achlorhydria. No characteristic bacteriologic flora for any of these diseases was observed.

Brunschwig considered the possibility that a bacterial toxin might be involved in the depressant action of gastric juice which he observed. However, when he used extracts of necrotic colon carcinoma, no depressant was found.

The significance of these findings in gastric surgery is obvious. Other than that, these results suggest that bacteriologic approach may be a sterile field for gastric cancer investigations in the present state of our knowledge. A possible exception might be a search for a filterable agent (virus) in gastric cancers. One difficulty in such a search would be that there is not yet available a large supply of such tissue from experimental animals.

CLINICAL ASSOCIATIONS

Atrophic Gastritis

One of the most frequently discussed associations of gastric carcinoma is with gastritis. Although this discussion dates back 125 years to the reports of Nepveu and Fenwick and runs through such exhaustive works as those of Konjetzny, Orator, Borrmann, Faber, Hurst, Puchert, and many others, it has not yet become a dead issue. Twenty-two major articles dealing directly with this problem were found in the literature of the past 5 years, as well as minor references too numerous to mention. The literature on this problem is difficult to summarize clearly by reason of its mixture with the literature regarding the association of gastric cancer with pernicious anemia and achlorhydria, both of which may be related to gastritis. For the purpose of clarification, however, it seems better to discuss the three problems separately.

Another complication is introduced by the fact that not all authors mean the same thing by the term "gastritis." In some cases where reports seem to be in direct conflict, it is evident that one author was speaking of an inflammatory process, another of a degenerative process, and perhaps a third of a mixture of the two. It is upon the degenerative and epithelial changes that most recent publications have focused, and it is in this sense that the word "gastritis" is used herein, in spite of the etymological difficulties. It would be of no

profit here to discuss the relationships of this form of gastritis with other forms, nor does this author feel competent to do so. Perhaps as Faber, Ewing, and F. A. Jones suggested, most, if not all, forms are manifestations of different stages in the same process. We see here one of the first needs in a coordinated attack upon gastric cancer, that is, the establishment by competent authorities of a better standard of nomenclature in order that collected data may be more easily interpreted and have more value for statistical studies.

Bennett said:

> The subject of gastritis is one very difficult to approach because the scientific physician feels that nothing is known of its etiology, its morbid anatomy is obscure, symptoms are often entirely absent, the prognosis is doubtful and, in fact, the one thing about which we can speak with certainty is the treatment—there is none.

Nevertheless, it seems important to try to evaluate our present knowledge and to attempt to formulate plans for an experimental approach to the problems involved.

Opinions vary widely between those who, like Konjetzny, Faber, and Hurst, consider that 80–90 percent of gastric cancers arise on a basis of gastritis and those, like Borrmann, who stated in 1926 that in early carcinoma he had almost never seen a severe gastritis. C. M. Jones, Turner, and Lieberthal, Stout, Guiss and Stewart, and Warren and Meisner all reviewed this subject in the past few years and concluded that the relationship was unproved and unknown. Their work shows that direct proof of a sequential relationship is lacking and, from clinical material, such proof may be far in the future. It would be profitless at this time to review the varying and sometimes opposing individual opinions and arguments upon this point. The reader who wishes to do so will find an extensive list of references in the bibliography. Briefly stated, the typical arguments are these. Some older workers thought that they could trace, by morphologic studies, all intermediate stages between gastritis and cancer. The more modern pathologists have studied more and perhaps better material and state that they cannot prove a connection between the two conditions. Certainly we have seen nothing in the literature that could be considered proof. We are also without information from experimental data. No one, so far as we know, has produced cancer on a basis of gastric atrophy. The gastroscopists are divided on the question. The positive and larger group led by Schindler considers that association of gastritis with cancer is so common that an etiologic relationship must be presumed. They do not, however, consider the case proved, and the consensus is that although the gastritis antedates the cancer the relationship may not be direct. As Schindler says, "the gastritis is only the soil" on which cancer arises. A smaller group believes that gastritis is a nonspecific reaction to injury and that the gastritis seen with peptic ulcer is the same as that seen with cancer. In the absence of a more dependable type of evidence, one must turn to clinical statistics.

As long ago as 1870, Fenwick and others called attention to the fact that atrophic gastritis could be seen in many conditions other than gastric cancer. Recently Guiss and Stewart, after careful examination and comparison of 304 stomachs from normals and from persons with various pathologic conditions, came to the conclusion that atrophic gastritis is an exceedingly common condition with advancing age and is frequently associated with conditions other than gastric carcinoma. Stout agreed with these conclusions. Their work indicated that gastritis is not present at birth, makes its first appearance in the second decade, and increases in incidence rapidly with age. They found gastritis in 82 percent of an apparently normal group of people over 40 years of age and in 97 percent of gastric carcinomas. One must agree with their view that it is difficult to prove by statistics alone a causal relationship for a condition that is found in 82 percent of normal persons. Nevertheless, a condition which is found in association with 97 percent of gastric carcinomas is to be viewed with suspicion. One may of course consider that the gastritis is the result of the carcinoma, in part at least (an opinion that Guiss and Stewart did not state, but others have). This, again, is an unproved point. Too many cases may be found in the literature in which the gastritis was known to antedate the carcinoma for us to exclude gastritis from the etiology of gastric cancer until better information is available than at present.

From the clinical standpoint, it is best to consider that, as Stout, Warren, and others have stated, the true relationships are unknown but some cancers may arise from gastritis, further that the great bulk of gastric cancers are associated with gastritis but that not all gastritis will lead to cancer. And most important, all known cases of gastritis should be carefully followed to detect neoplastic changes. It is very important to collect more and better data upon the clinical course and sequelae of atrophic gastritis. Collection of the data at some central point is to be preferred. A program for the careful and coordinated study of gastritis and other conditions in relation to gastric cancer was set up in Cincinnati by the late Dr. M. R. Reid. Such endeavors are of great value and should be multiplied and extended.

Jankelson and co-workers followed 18 cases of primary atrophic gastritis for 2 years and found that in 2 cases a polyp had developed. It seems reasonable to assume from statistics that if all cases

of gastritis were carefully followed from inception something like 80–85 percent of gastric cancers would be discovered. But this is of little help at our present state of knowledge because, in the first place, most cases of gastritis are not diagnosed and, in the second place, as has been shown, we should need to follow 80 percent of people over 40 years of age, an impractical undertaking.

As a problem for animal experimentation, this offers an almost unexplored field, and it is probably from this type of work that the final answer must come. What is needed is a good reproducible method of producing atrophic gastritis in a suitable animal. In saying this, we are echoing the consensus of the gastric cancer conferences held in 1940 and 1944. There is no report of the spontaneous occurrence of gastritis in animals. Goodpasture made an extensive study of senescence in the dog: but although a few benign tumors of the stomach were found, he did not mention gastritis.

Since Beaumont's time, numerous attempts have been made to establish alcohol as an etiologic agent in gastritis. Of course, it is relatively easy to produce acute and transient changes by means of alcohol, but there seems to be but one report of severe atrophic gastritis produced by alcohol experimentally. That was the report by Thomsen and concerned only one dog. A good review of this subject can be found in the essay of Beazell and Ivy. They concluded that

> until crucial experimental evidence is available it must be accepted as a possibility that the impaired gastric secretion associated with alcoholism may be due in part to a nutritional deficiency.

Two good clinical studies of the stomachs of chronic alcoholics by gastroscope appeared in 1941 (Gray and Schindler and Berry). Surprisingly little gastritis was found, especially so when one considers the extremes of abuse and nutritional deficiency to which the stomachs of such patients are subjected.

Other dietary irritants, drugs, irradiation, allergic stimuli, and transplantation of tissues have been tried, but most experiments were not carried on over extended periods, the animals died of acute reactions, or results were inconclusive. The most encouraging results seem to have been attained with irradiation. Ivy reviewed these matters at the last gastric cancer conference, and the reader can find a complete discussion of the physiologic aspects of the problems in the report of that meeting.

There have been suggestions from the clinical literature that various endocrine glands might be involved. Of these the hypophysis and thyroid seem most encouraging. Magruder reported that in the stomachs of hypophysectomized dogs he had observed "lymphoid accumulations and loss of glandular tissue."

The suggestion of Shapiro et al. that gastritis may be a deficiency disease deserves further investigation. They reported evidence which suggested that choline might have a curative effect on gastritis. Several reports of regeneration of the gastric muscosa under liver therapy are found in the literature. The evidence is not conclusive. It seems desirable to have more correlated gastroscopic and microscopic studies in these experiments with the therapy of gastritis.

We conclude, then, that the literature does not supply the desired facts in regard to experimental production of gastritis, and that generally speaking there are no closed doors to future investigations.

Pernicious Anemia

Of the several divisions into which this review falls naturally, the possible relationship of gastric cancer to pernicious anemia seems to excite a disproportionate amount of literary effort. Five major articles on the subject appeared in 1945, and during recent years over 80 important articles touching upon the subject were published.

Rhoads in 1941 reviewed this subject and concluded that the occurrence of gastric cancer in persons having pernicious anemia was more frequent than could be explained by the laws of chance. Other more recent studies have confirmed this view. A good example of these is the study of Kaplan and Rigler.

The evidence rests entirely on a series of clinical observations. Briefly, the apparent facts are these: A search for gastric cancer in patients with pernicious anemia will reveal an incidence which is much higher than would be expected in the same age group of the general population, and in addition an abnormally large number of gastric polyps. If one may judge by the series of reports emanating from the Mayo Clinic, this incidence may be rising as more and more people with pernicious anemia are permitted, by modern methods of therapy, to survive for a number of years. It has even been suggested that the injection of liver extract may have an influence on the development of cancer in patients with anemia. One supposes that such a suggestion is based in part at least upon the reports that subcutaneous sarcomas may be produced in mice with liver extract (Steiner). It may be quite misleading to assume that a locally active carcinogen has also a remote carcinogenic effect.

When the concurrence of the diseases is viewed the other way around, the correlation is not so clear since there are few series in which the number of cases reviewed is large enough to satisfy statistical demands. However, there are indications that if cases of gastric cancer are surveyed for pernicious anemia, there is again more than

726906—47——5

the expected incidence. A similar high incidence is not found when cases of cancer of the large intestine are surveyed for pernicious anemia (Kaplan and Rigler). From another viewpoint, the correlation is better; in patients having pernicious anemia and a neoplasm, the great bulk of the tumors are in the stomach (e. g., Cotti, 86 percent; or Kaplan and Rigler, 60 percent).

It seems that until a better type of evidence becomes available, we should assume as a working hypothesis that there is some relationship of unknown nature between pernicious anemia and gastric cancer and that people with pernicious anemia are subject to a greater hazard in this respect than is the general population.

The etiologic relationship, if any, is generally assumed to be by way of atrophic gastritis, which is regularly encountered in pernicious anemia and nearly always accompanies gastric cancer. Velde states that he has seen progression through the sequence gastritis, achylia, pernicious anemia, gastric cancer.

In most cases the pernicious anemia precedes the cancer. Intervals up to 15 years are reported. The reverse has been reported rarely.

That the association of the two diseases may be on a hereditary basis is indicated by several reports of close association of achlorhydria, pernicious anemia, and gastric cancer in family histories. However, one must be wary in accepting such evidence.

The exact character of the macrocytic anemia which may accompany gastric cancer is not known. Oppenheim and his colleagues gave good reasons for believing that it is not pernicious anemia. Apparently both hyperchromic and normochromic varieties may be seen. Further knowledge of these anemias would be very desirable.

The important consideration here is, if there is a relationship between pernicious anemia and gastric cancer, whether direct or indirect, how can the information at hand be used. The obvious use is in cancer control. One should watch the stomachs of patients with pernicious anemia for the development of cancer or polyps, with more than ordinary care. Such observations should reveal more gastric cancers than would be obtained from an equal number of the general population. This is being done in some quarters. Enlargement and extension of the program would probably be well advised. However, one should not expect too much from such efforts, because of the following considerations.

Two thousand three hundred ninety-six people died from pernicious anemia in the United States during 1943 according to Census Bureau statistics. If one takes this figure as a rough approximation (admittedly inaccurate in several respects) to the annual incidence of new cases and applies to it one of the highest figures for coincidence with gastric cancer, e. g., 12.3 percent (Kaplan and Rigler), the expected number of gastric cancers is 295. When this figure is compared with the total number of deaths from gastric cancer it is seen that we might expect to find just over 1 percent of all gastric cancers by such means. Thus, while this aspect of a control program is worth while, it cannot be expected to produce a marked change in the overall situation.

From the experimental side, there is not much to be said. Clinical investigations now being carried on may yield better information regarding the statistical aspects discussed in the beginning of this section. They may also uncover useful information in regard to the therapeusis or pathology of the associated gastritis, either one of which might conceivably throw some light on our problems in gastric cancer. The controversy regarding regeneration of the mucosa during liver therapy is particularly interesting. Observers have variously reported almost complete regeneration, no regeneration, or regeneration of only the superficial layer. There is rather general though not complete agreement that the hydrochloric acid does not return. What seems to be needed to settle this controversy is more combined gastroscopic and microscopic studies.

Various dietary studies may have a bearing on this phase of the gastric-cancer problem, but they are discussed in other sections of the review.

It would be valuable if, incidental to the studies of anemia, one could discover means of differentiating the macrocytic hyperchromic anemias that may be secondary to gastric cancer from those that are primarily pernicious anemia, if, indeed, there is any difference.

We have not considered here the experiments done on the reverse aspect of this problem, i. e., attempts to produce pernicious anemia through gastric deficiency.

Achlorhydria

The relationship of achlorhydria to gastric cancer has been the subject of a great many articles. While there are in this field, as elsewhere, differences of opinion, in general the differences are not so irreconcilable as in the case of gastritis or ulcer. It is not too difficult to arrange the material in reasonable order and to draw some conclusions. This has been done so well by Ivy recently that we shall merely summarize here and make a few additions.

The literature reviewed contains only two noteworthy recent additions to those cited by Ivy. Chitre reported a small series of cases from India which gives further evidence that there is no apparent racial difference in the association of achlorhydria with cancer. Wolman reviewed the litera-

ture in regard to achlorhydria in childhood. He found one case of true achlorhydria in a 10-year-old girl "with malnutrition" whose mother had pernicious anemia. This is the earliest case that we have seen reported, and its familial association with pernicious anemia makes it doubly interesting.

The literature may be epitomized thus: Achlorhydria (true or histamine-fast) is a common condition. It is not present at birth, tends to appear in early adult life, and increases in incidence steadily to old age. In this regard it resembles gastritis, but the incidence is much less. The observations of Vanzant and co-workers indicate that there is more than one factor involved in the increase in the incidence of achlorhydria that occurs with aging. The nature of the factors is unknown. Unlike gastric cancer, achlorhydria is somewhat more common in women than in men. Achlorhydria like gastritis is a frequent concomitant of diseases other than gastric cancer. Nevertheless, more achlorhydria is found in association with gastric cancer than would be found in a group of the same age from the general population. Twenty to twenty-five percent of the general population of gastric cancer age have achlorhydria whereas gastric-cancer patients have an incidence of more than 50 percent, and in addition a large number have hypoacidity. An average figure from the literature for incidence of achlorhydria in gastric-cancer cases is about 65–70 percent. In the series of 501 cases of gastric cancer in the young, collected by McNeer, there is also a marked divergence from normal. Fourty-four percent of the cases were achlorhydric. In the same age group of normals, one would expect about 5 percent to have achlorhydria.

The controversy whether the achlorhydria precedes or follows the carcinoma seems to have been resolved, insofar as case reports can do so, in favor of both sides. There are several recorded cases in which acid was observed to decrease or disappear after the presence of a cancer was established. There are also many recorded cases in which the presence of achlorhydria was discovered many years before the appearance of other signs of cancer. The objection might be raised that in the latter group there may have existed an otherwise symptomless cancer for a considerable time. In the light of our present knowledge of the natural history of gastric cancer, it seems very improbable that this could have occurred in any considerable number of patients. Attempts to correlate achlorhydria with the type, location, or size of the tumor have not succeeded.

The connection between achlorhydria and gastric cancer is probably indirect and possibly multiphasic. It seems reasonably clear that in many cases, if not most, the connection is by way of gastritis. However, from the work of Faber, Schindler, and others, it seems that achclorhydria occurs in the absence of gastritis and that gastritis is seen without achlorhydria, although, of course, a completely atrophic mucosa cannot secrete acid.

In considering possible relationships one must keep in mind that humoral as well as anatomic factors may be involved. Brunschwig and co-workers reported a secretory depressant in achlorhydric gastric juices. Ivy suggested the possibility that in the presence of intestinal metaplasia enterogastrone may be present in the stomach. Phillips and others reported that an increased power of neutralization may be observed in carcinomatous stomachs.

The relationship of achlorhydria to other conditions is undoubtedly complex, and complete understanding does not seem possible at present. However, a relationship of unknown nature between gastric cancer and achlorhydria appears probable. Until further knowledge is available, a certain amount of use may be made of the foregoing indications.

The most obvious application of the available knowledge is to a cancer-control program. On a statistical basis, it is evident that, if one-fourth of persons of cancer age have achlorhydria and approximately two out of three gastric cancers are associated with achlorhydria, the distribution is disproportionate, and some sort of "concentration factor" must be operating. How this comes about we do not know. It appears, however, that it might not be too difficult to segregate large groups of people over 50 years old having achlorhydria. A systematic follow-up of such a group, employing among other things fluoroscopy could be expected to yield much valuable information. In the course of time one would expect to find gastric cancer in a ratio of about 1 cancer in 150 to 300 patients, based upon incidence rates recently published. This seems worth while. Bloomfield and Polland followed cases of unexplained anacidity for from 1 to 7 years and encountered no cancer and no pernicious anemia. There were but 45 cases in their series, and normal expectancy would suggest that the group was much too small to give interpretable results. If such methods are to produce results, they will have to be so designed as to give proper consideration to mathematical factors.

The significance of achlorhydria in diagnosis is mentioned later under that heading. In relation to prognosis, Hartman many years ago made the interesting suggestion that the 5-year-survival rate appeared to be better in those cases with a resectable lesion and without free hydrochloric acid than in those with free acid. This was not borne out by the larger series of Comfort and Vanzant, although the two statements are not in direct opposition. Comfort and Vanzant studied over 800

cases and concluded that resectable lesions are associated with a higher acidity and a lower incidence of achlorhydria. One might guess that when acid, especially in high concentration, is present, pain is experienced sooner and more persistently and that this would drive the patient to a physician early while the lesion is still resectable. Such a rationalization would explain the observations of Comfort and Vanzant. This does not decide whether the prognosis is better or worse for a given extent of lesion in the presence of achlorhydria. An analysis of cases matched for extent of lesion should be made to decide this interesting question. Again and again one is struck by the fact that even the more detailed reports of clinical observations now available leave out, because of their special focus of interest, much information that would be useful. This condition will probably persist until such time as an organized effort is made to improve and to a certain extent standardize the collection of data.

As a problem in animal experimentation, achlorhydria seems to have many aspects. From the standpoint of cancer research, it is probable that at present the most important phase is that in which the problem is blended with the production and study of atrophic gastritis. The development of achlorhydria, gastritis, pernicious anemia, and gastritic cancer may depend upon some common antecedent condition which leads in some cases to one or another of these and in some cases to all. The processes that cause a reduction in secretion after the establishment of a cancer may not be the same as those that cause the achlorhydria that precedes a cancer. Vanzant and her co-workers believed that their data indicated that more than one factor was operating to produce a lowered acidity throughout the lifetime of a large group of normal people. The very large amount of work that has been reported on attempts to reduce the acidity of the stomach in the therapy of peptic ulcer cannot be included here except for particular details. A good introduction to this literature may be found in the recent review of Palmer and the paper by Atkinson and Ivy. The most interesting leads with reference to the problem here are the reports of Brunschwig and co-workers on a secretary depressant found in gastric juice and present in high concentration in the achlorhydric juices from stomachs of patients with pernicious anemia or gastric cancer and the reports on enterogastrone and urogastrone by Ivy and co-workers. The temporary achlorhydria accompanying the acute gastritis produced by hot water and X-rays is also very interesting and suggestive, as are also the reports of the depression of acidity produced by hormones and vitamin deficiencies. Cowgill and Gilman observed an achlorhydria refractory to histamine in one Pavlov-pouch dog that was markedly deficient in vitamin B_1. Two other dogs in the same experimental group remained normal. Schnedorf and Ivy observed spontaneous achlorhydria in monkeys. Oddly, the monkeys later responded to histamine after having been given mecholyl. This suggests that the monkey might be a good subject for investigations designed to produce experimental achlorhydria. More work along these lines may lead to a reproducible method of producing achlorhydria; and a valuable tool may thus be furnished to workers who wish to study the achlorhydric stomach in relation to cancer. Naturally, such investigations would also be of the greatest interest in relation to peptic ulcer and pernicious anemia.

Ulcer

The literature on gastric ulcer has staggering proportions, but only a small fraction of it deals with cancer. We shall confine our review to the latter category. Ewing said in 1940:

> It is evident that the relations between ulcer and cancer of the stomach are rather close, quite obscure and no complete explanation of these relations has yet been provided.

As in the case of atrophic gastritis, the literature regarding the development of gastric cancer in gastric ulcer contains extremes of opinion. There are those who believe, like Wilson and MacCarty, that 71 percent of carcinomas originate in an ulcer, and those who, like Schindler and Hinton, believe that a benign ulcer almost never becomes malignant. The great majority of authors express an opinion between these two extremes and say that 10–20 percent of gastric cancers arise in a benign ulcer. The morphologic evidence that cancer can arise in an ulcer is well-known. How often that happens is more difficult to determine. Schindler states that in cases of gastric ulcer which he has followed gastroscopically over a period of several years, he has never seen carcinoma develop.

The danger of malignancy seems to be greatest in ulcers of the greater curvature and least in ulcer of the lesser curvature. Mallory called attention to the diagnostic difficulty which arises when cancer in the preinvasive stage becomes ulcerated, and more recently Easterman and Palmer and Humphreys reported observations emphasizing the same thing. Allen wrote one of the most recent surveys of this problem and its implications in diagnosis and treatment. For the purposes of this paper, it is sufficient to say that apparently gastric cancer can arise from ulcer, but there is no experimental proof that it does so.

As a problem in cancer control, it is suggested that the physician might do well to be more alert than ever before. The report from the Massachusetts General Hospital by Allen in 1945 and the reports by Judd and Priestley in 1943 and Kier-

nan and Larson in 1946 from the Mayo Clinic as well as earlier reports by others should convince anyone that the risk of a mistake in diagnosis is a very great one, and that no one of the present modern steps in diagnosis short of microscopic examination to the tissue, not even actual inspection and palpation of the lesion at operation, is adequate assurance that a given lesion is benign. Furthermore, statistics seem to indicate that the actual mortality risk is greater in medical management than in radical surgery in that group of cases in which diagnosis may be in doubt. The wider realization of these facts may serve to bring some gastric carcinomas to the surgeon earlier and at a time when the possibility of cure is greater. A fair yield of gastric cancer might be anticipated from a control program designed to discover early cancer in patients with gastric ulcer: about 1 cancer in 10 patients might be expected, but this method would discover only about 20 percent of all gastric cancer, perhaps less.

The suggestion for clinical research seems to be one of follow-up study and collection of clinical evidence. The follow-up studies reported do not seem to include a sufficient number of patients to permit definite conclusions or in some reports to cover a sufficiently long time. In this group, the more frequent reporting of gastric analyses might be of some help. One frequently sees in the literature case reports which give the impression that a better understanding of the relation of the case to the larger picture might be had if it were known whether this case was one of the minority that show considerable free acid or belonged in the larger achlorhydric group. There is urgent need for some diagnostic method that would help differentiate the gastric cancers that masquerade as ulcers from benign ulcers. No suggestion was found in the literature which indicated the direction from which such help might be expected nor indeed if it might be possible at all. Nevertheless, the need exists.

For animal experimentation, the outlook is somewhat clearer than in chronic gastritis. The great variety of methods for producing ulcers in experimental animals is too well known to require reiteration here. To produce cancers in these ulcers may be more difficult. The application of carcinogenic chemicals to experimental ulcers, either directly or by feeding experiments has already been suggested by Hollander, Ivy, Voegtlin, and others. Ivy has suggested that perhaps the rabbit may be a good animal for this work because of the adenomatous overgrowth that is seen in healing ulcers in this animal. The mere maintenance of sufficient chronicity in the lesion may also produce the desired result. Perhaps the failures reported in these experiments are due to the short interval of time, and methods already used may succeed if we recall the lesson learned from the experiments in which tar was painted on the ears of rabbits to produce carcinoma.

Little will be said regarding duodenal ulcer. The opinion seems almost universally held that a duodenal ulcer never, or very seldom, becomes malignant. We would expect such a change to be possible, but it must be very rare. The proof of this rests entirely on clinical statistics, a foundation which may not be trustworthy. The relative immunity of the duodenum to neoplasia when compared with the immediately adjacent stomach is a mystery for which there is no known explanation.

Gastric Polyps

There is wide agreement that gastric polyps are precancerous. There has not been experimental proof of this potentiality, although some pathologists have said that they could trace the development of malignant changes in polyps morphologically. Just what percentage of them become malignant is not agreed, and estimates vary from 12 to 50 percent. Their importance in our considerations here is minimized by the fact that polyps are not very common, and no large proportion of total gastric cancer can be accounted for on this basis. Best estimates seem to be from ½ to 1 percent.

The literature on this subject is not large. Pearl and Brunn in 1943 published the most recent review of the subject that we have seen. The critical facts gleaned from the literature are these: Gastric polyps are rare, but the frequency of diagnosis is increasing. They are more common in the male and occur at about the same age as cancer. There may be a congenital and an acquired type. They are frequently associated with atrophic gastritis and pernicious anemia. Most cases are achlorhydric. Polyps have been observed for years without malignant change, but Rigler and co-workers state that they have seen a rapid change from a benign polyp to a cancer in their patients with anemia. There seems to be little dissent from the opinion that surgical removal is indicated.

Their place in cancer control has already been indicated to be a small one. Any program that involves X-ray or gastrocopic examination of groups of people past middle age will uncover some cases of polyposis. If these are removed early, some gastric cancer will be prevented.

As a matter for clinical investigation, this phase seems to offer only limited scope. Nevertheless, close observation of these cases might throw some light on the more general problems of gastric cancer and is not to be discouraged.

In animal experimentation, this field offers one unique advantage. Old dogs may develop gastric polyps spontaneously. Adenomatous and polypoid lesions occur spontaneously in some mice, and

strain I mice characteristically develop an adenomatous hyperplasia. Here, then, is one case in which the experimental animal presents a ready-made tool, something not often encountered in gastric-cancer research. The possibility of inducing malignant changes in such lesions must have occurred to many observers. Perhaps it should also be mentioned that polypoid and adenomatous lesions have also been reported to be induced in rats and mice by nutritional deficiencies, particularly vitamins, by parasites, and by the presence of mechanically irritating foreign bodies. None of these latter endeavors has proved very productive to date, but much might yet come from such studies.

DIAGNOSIS

A survey of the recent literature on the diagnosis of gastric cancer suggests our wartime phrase "too little and too late." The record is not a good one, and it has shown little improvement in recent years. A share of the responsibility for this can be ascribed to each of the various elements involved from the inception of the disease to the arrival of the patient in the operating room or the morgue. The insidious and often symptom-free onset of the disease accounts for the first loss of time. The patient is tardy in visiting the physician after the first vague symptoms are noticed. The physician is often slow in suspecting the disease, in either the patient or himself, and in getting the case to the gastroenterologist or surgeon. The delay by the physician is blameworthy only in part. There is no typical syndrome for gastric cancer from which he may make a diagnosis.

The descriptions of the disease found in many textbooks are applicable only to the late stages and are of little use in finding the disease while there is yet time for a cure. The diagnostic rule of thumb may be wrong or misleading. For example we have encountered these: "Where there is acid there is no cancer." "Gastric cancer occurs in the gastric athlete." The first is wrong in many cases, and the second is misleading because a fourth or more of cases will give a long dyspeptic history. Viacava and Pack have shown that invasion of the supraclavicular node is present only rarely (about 2.8 percent of cases), and then only in advanced stages of the disease. There does not seem at present to be any easy and dependable aid to the diagnosis of early gastric cancer upon which the general practitioner can rely.

If the lesion is still in a resectable condition when the patient arrives at the clinic, the situation is much better. The roentgenologist can make an accurate diagnosis in a high percentage of cases. There are some cases that he will miss altogether; and of those that appear roentgenologically to be gastric ulcer, approximately 10 percent will prove to be cancer. The advent of the gastroscope has materially improved the situation in recent years. This instrument has not been in general use a sufficient number of years to produce many reports from highly trained men. It appears from the reports available that from 80 to 90 percent of satisfactory diagnoses are being obtained. The cases missed are not always the same ones missed by X-ray, and the two working together will usually make a correct diagnosis. The residuum of undiagnosed gastric cancer among the patients of a good clinic is small and of little consequence when viewed against the background of the problem as a whole. So we return to the means of early diagnosis available to the general practitioner.

There is an imposing array of opinion which holds that the most important single early sign of gastric cancer is anacidity or hypoacidity. The impression gained in this review is that this is probably true, but it is not necessary or wise to rely upon a single criterion. The series of cases reported by Holman and Sandusky are interesting in this regard. Most authors have reported in their series a small group (about 10 to 20 percent) of patients having cancer and considerable free acid. It is this group of cases that is most apt to be difficult to diagnose. The statistical analysis by Comfort and Vanzant demonstrated clearly that there are cases in which it is not possible to make a differential diagnosis with the present methods of gastric analysis. At the risk of making a confused situation more confused, we should recall some anomalous facts. Achlorhydria occurs in the presence of gastric ulcer. Some gastric-cancer patients with free acid have ulcer symptoms and some do not. To make matters worse, some gastric-cancer patients with achlorhydria have ulcer symptoms. So it becomes apparent that there is so much overlapping between various groups that clear-cut diagnosis of a given case may not always be possible. Even the surgeon who sees and feels the lesion at operation is also sometimes wrong in diagnosis. Clinics specializing in gastroenterology can differentiate some of these lesions, but it appears that at present only a microscopic diagnosis is certain in all cases. No suggestion was found in the literature as to how errors in diagnosis may be avoided in the group of cancers that appear to be ulcers and may even show roentgenologic signs of healing under ulcer management. Further knowledge gained in the clinic or laboratory seems to offer the only solution to this problem. It has been frequently suggested that apparently normal persons of cancer age should be routinely checked by X-ray for gastric cancer in the hope of finding early cases. There are two recent reports of searches for gastric cancer among

apparently normal individuals of cancer age. The examination of 2,413 people by St. John et al. yielded 2 unsuspected cancers, 1 lymphosarcoma, 1 polyp, 20 ulcers, and 527 other benign lesions. This work is also interesting as to technique. They are using a method of rapid fluoroscopy that reduces the cost of such surveys in time and materials to a minimum. Dailey and Miller examined 500 apparently healthy men over 45 years of age and found 3 benign lesions but no cancer, and concluded that such surveys were not worth while. Based upon incidence rates reported by Dorn for 1944, the expectancy in such a group of 500 would be only 0.7 cases. Therefore one would need a larger group for evaluation of the method.

There are some very informative papers regarding the risk that is run in medical management of gastric ulcer. The article by Judd and Priestley is one of these. It is quite possible that in selecting the more conservative management one may be taking a greater risk than would have accompanied radical treatment.

Some encouraging reports are to be found with regard to diagnosis by examination of smears of cells from gastric contents after the manner of Papanicolaou. Kenamore et al. recently reported good results in obtaining biopsy specimens with a special instrument. It would seem best to withhold judgment on such methods until further reports and possible improvements in techniques are available.

The literature since 1940 contains references to seven innovations in diagnosis of gastric cancer. These involve decolorization of methylene blue (Chamorro-R. Salinas), an electrogram (Kozawa et al.), a dietetoquimogram (Sainz), a physicochemical method involving the pH, viscosity, surface tension, and refractometric index of the gastric juice (Cytronberg), a method involving the determination of the vitamin C content of the tissues of the stomach (Iacobovici et al.), the electro-cardiogastrograph (Feros), and the pattern formed by a dried drop of blood on a glass slide (Bolen). We saw no reason to believe that aid in diagnosis can be expected from any of these methods at the present time. One cannot say what future work may reveal in regard to any of them. The most interesting at present is the electrical method. Rehm and Bozler recently made fundamental studies in this field, and Hollander reviewed the subject. Feros reported his attempts to evaluate the method in clinical material. Basically the method depends upon the determination of variations in the intragastric electrical potentials by an electrocardiograph or similar instrument. There are a priori reasons for expecting valuable information to accrue from such studies even if no method of diagnosing cancer results, and we shall follow further developments with interest.

On completion of this section of the review one is left with the feeling that there is much to emphasize the gravity of the situation but little to suggest a possible solution of the difficulties. Good results can be expected from the several educational campaigns that are being carried on at present. Perhaps, if only by stimulating a more alert and suspicious attitude on the part of the physician, general knowledge of the situation may bring about a marked increase in the number of early cases receiving adequate attention, even with the poor methods of diagnosis now available. That, however, is not our concern here. We are searching for suggestions for experimental investigations.

The conviction has been strengthened that one of the most pressing needs is some addition to our methods of early diagnosis. No good suggestion was found. Cytologic studies of the gastric contents or biopsy methods seem to be the most hopeful at the present moment. Further pursuit of the question of electrical potentials might lead to something. Studies of the mucus or other constituents of the gastric juice already mentioned may give a lead. Increased knowledge of the true relationships of achlorhydria, pernicious anemia, or gastritis might reveal new diagnostic criteria. Even if these were not direct, it would help a great deal if one could learn to concentrate diagnostic efforts on some relatively small but more susceptible section of the population.

Certainly this phase of the problem is as unknown and wide open for investigation as any other phase. Significant leads are unfortunately even fewer than in most aspects of the problem.

EXPERIMENTAL CARCINOGENESIS

The reviewer is fortunate in being able to begin by reference to the review by Klein and Palmer (1940). Their review, which is the most comprehensive single article on the subject, covered all the early attempts in this field and concluded that up to that time there was no well-established case of the experimental production of gastric adenocarcinoma in animals reported. They reviewed the many articles upon the production of squamous-cell lesions of the forestomach in mice and rats and found no reason for believing that such lesions are analogous to the glandular lesions in the human stomach. We concur in this view and as stated before are not considering such lesions in this paper. Their discussion of criteria of malignancy and of factors which may have had an influence in the failure of some experiments should be read by all who plan experiments in this field.

Since 1940 two important new facts have been established. The glandular mucosae of the mouse and rat have been shown to be susceptible to extrinsic carcinogens, and a strain of mice said to have spontaneous adenocarcinoma of the stomach

has been developed. Rusch and co-workers injected benzpyrene into the wall of the pyloric chamber in 5 rats and reported 1 "low grade adenocarcinoma" without metastases that occurred after 15 months. No illustration or description of the tumor was given. Stewart and Lorenz observed several undoubted adenocarcinomas in mice following the injection of methylcholanthrene dispersed in horse serum, into the stomach wall. Their paper is well illustrated, and meticulous descriptions of the tumors are furnished. The tumors were transplanted through 11 generations. No metastases were observed, but the tumors can be readily accepted as malignant. Tumors were obtained in 3 strains of mice, C3H, I, and C57 brown. One tumor was obtained when mineral oil was used as the vehicle. Dosage of the hydrocarbon varied from 0.4 to 0.12 mg. Latent periods varied from 19 to 46 weeks. The war interrupted the work in its early stages.

The production by Strong of a strain of mice said to have spontaneous gastric carcinoma is a contribution of the first order. However, not all workers agree that the lesions are malignant. Apparently the strain is not sufficiently inbred at the present time for complete evaluation, but it appears that the incidence of gastric lesions will be high. For the details regarding the production of the strain, the reader is referred to the original publications.

These two reports materially alter the outlook in experimental gastric cancer, and the work should go forward more rapidly in the future. There are some further additions to the literature since 1940. Many of these have to do with the feeding of heated fats and cholesterol derivatives and have already been mentioned in the section on diet. The summary in tabular form by Sugiura is a useful supplement to other reviews.

Roffo reported lesions in the stomachs of rats fed bread and milk plus tobacco tar or fuel-oil distillate for long periods. The same criticisms may be applied to these reports that were applied to the earlier work with heated animal fats. One must leave the question open. Dickens and Weil-Malherbe reported negative results when smoked fish or meat was fed to rats for periods up to 20 months. Otherwise there is little change since the Klein-Palmer review. Advances in our knowledge of the suspected precancerous or associated lesions have already been noted in appropriate sections of this paper.

It is obvious that much work remains to be done. What avenues of approach are open? Several have been suggested by the material covered in this review. Few if any appear to be broad and inviting or to offer easy success. The outlook, however, is not forbidding, and the opportunities for the acquisition of important contributory facts along the way are many.

It seems that in many cases our immediate goal should be something short of the actual production of cancer in the stomach of the experimental animal. Much may be accomplished by a study of subsidiary problems. Acquisition of new facts that appear superficially to be related only to gastric physiology could be of the greatest use in cancer work. One has only to remember the tremendous influence that the newer facts in relation to endocrinology has had on the study of cancer of the breast and prostate to see that this may be true.

As susceptible strains of animals become available, all the encouraging leads in the older literature should be re-examined with respect to these susceptible animals. This is particularly true of the dietary factors and the substances related to bile. The carcinogenic chemicals should be resurveyed to discover if there is one or a class that is especially active in the stomach.

When success has been achieved in producing gastric cancer in one species, there is encouragement to try further with other species. It would be especially valuable if positive results could be obtained in a species having a stomach more nearly like that of man.

Attempts to produce chronic atrophic gastritis, achlorhydria, chronic gastric ulcer, etc., are of major importance. The most encouraging leads obtained so far in this line are the effects seen with hot water, alcohol, or X-ray.[1] If experiments along these lines succeed, valuable material will be obtained with which to go on in the study of the etiology of gastric cancer.

In susceptible strains of animals subjected to carcinogenic stimuli, observations should be made to determine whether any of the conditions associated with gastric cancer in man are seen in the animal. This of course entails the use of large numbers of animals and routine examinations during the latent period.

Some of the more hopeful procedures for inducing adenocarcinoma should be tried in rabbits having adenomatous overgrowth in ulcers.

It is not unlikely that in all these animals a major stumbling block is our lack of knowledge of the physiology and spontaneous pathology of the stomach in the particular animal used. For this reason, basic studies in physiology and pathologic physiology may be one of the more informative lines of investigation. This applies especially to rodents which will be used in many of the experiments.

Procedures designed to produce ulcer should be modified where possible to promote greater chronicity. Many experiments have been carried on for short periods only. Latent periods may be long in

many cases especially in the case of the dog, monkey, and rabbit. It was at one time thought that the rabbit did not have mammary cancer. Extended observations showed that this is not true.

MISCELLANY

Mucus is concerned in gastric-cancer studies for several reasons. The facts available are few, however, and somewhat scattered. Like so many other angles of the gastric-cancer problem, this one requires a good real of tolerance and speculation.

The physiology and chemistry of gastric mucin are not well-known, and it is quite possible that the most valuable contributions to this phase of the gastric-cancer problem will come not from a study of cancer but from basic physiologic investigations.

Mucous cells and mucus or related substances are a prominent feature in many neoplasms. Their relations there are not known. Popoff showed that in the colon the mucous cell undergoes a cyclic regenerative cytomorphosis. He suggests that at certain stages this might be diverted into neoplasia.

Most of those who have studied mucus have looked upon it as protective and lubricating in function. The application of various irritant substances to the gastric mucosa is one of the methods of stimulating mucus secretion. This is generally interpreted as a protective response of the mucosa. Wolf and Wolff, in their studies of the human stomach, observed that mucus precipitated on and clung to an injured area. In the absence of mucus, an ulcer was formed where injured mucosa was exposed to gastric juice. If chronic irritation or invasion by external agents is important in carcinogenesis, then a protective layer such as mucus becomes important. Many now seem to believe that in its role as a protective barrier to external carcinogenic agents mucus reaches its greatest importance in gastric cancer. The failure of all attempts to induce gastric adenocarcinoma by feeding carcinogenic chemicals to mice and rats is usually explained in this way. The exact mechanisms that may be involved are not at present known. There is nevertheless a considerable body of competent opinion that assumes an importance for the mucus which would warrant intensive investigation. Hollander and Ivy each recently discussed this aspect of the gastric-cancer problem, and Babkin reviewed the physiology of mucus secretion.

Another point of interest in regard to mucus is in relation to atrophic gastritis. Almost all observers emphasize the prominence of metaplasia toward the intestinal type of epithelium with an increase in the number of mucous cells. Mucous cysts are frequently seen, and Judd expressed the opinion that the fundamental difference between the stomach with and the one without carcinoma is the relative lack of mucous-cell hyperplasia in the latter. Guiss and Stewart found that intestinal metaplasia, pyloric gland heterotopia, and mucosal cysts were more prominent late in life. So it seems that whatever the true nature of the relationship (and it may prove to be of no significance on further investigation), the mucous cell is found in an interesting association with gastric cancer, with the cancer age, and with one of the suspected precancerous lesions.

Another interest in mucus arises from the chemical nature of the material. Here again the thread of connection is thin. Gastric mucin has considerable neutralizing power. Hollander recently reported pH values around 7. The buffer capacity of some specimens is as high as 84 mN. Opinions vary regarding the relative importance of neutralization by mucus in the stomach, but most agree that neutralization is one function of mucus. A few observers have said that an increased power of neutralization may be found in the carcinomatous stomach. It has been shown that mucus has antipeptic powers and that it is penetrated slowly by pepsin, hydrochloric acid, and dyes.

In the functional derangement most commonly associated with gastric cancer, i. e., achlorhydria, the mucous cell may have an importance that is not yet apparent. The neutralizing and buffering properties of mucus have already been mentioned. No excessive secretion of mucus in achlorhydria has been reported. In those attempts to produce achlorhydria which resulted in a temporary effect or partial success, an agent affecting the mucous cell was often employed. For example, silver nitrate, hydrogen peroxide, and alcohol all stimulate mucus secretion. X-ray has a differential effect on the mucous cell, it being relatively late in degeneration after irradiation.

For the sake of completeness, it should be pointed out that mucus has a more or less remote connection with the three other conditions most commonly mentioned in association with gastric cancer. In relation to ulcer, there is the observation noted previously that ulceration occurred in an area free from mucus and there are numerous reports of successful ulcer therapy with mucin.

In relation to polyp, the mucous cell is prominent in the histology of polyps, and Pearl and Brunn have called attention to a myxorrhea that may be observed in multiple polyposis gastrica. It seems a little farfetched, but in relation of the anemias there is the observation of Wigodsky et al. that commercial gastric mucin stimulates hemoglobin formation in the dog more than can be accounted for on the basis of the iron contained.

It has been suggested that an investigation of the chemical nature of the mucoid material in gastric cancer might lead to the discovery of some

growth accelerator. Williams reported that when tested on a lymphosarcoma transplanted into C3H mice commercial gastric mucin from the pig inhibited the growth of the tumor. The effect was ascribed to an augmentation of the host's defensive reaction. This is an interesting contrast with the frequently reported "aggressin" like activity of mucin when bacteria are suspended in it before injection into an animal.

There seems to be little of significance in the foregoing for application to clinical lines at present (we are not discussing ulcer, of course), but there are strong suggestions for much work along fundamental lines. The main objective is the acquisition of basic knowledge that may prove of use in cancer work, rather than a direct attack upon experimental cancer.

There is some interest in the possible role of bile in the pathogenesis of gastric cancer. Ivy discussed this in 1945.

Perhaps the greatest interest centers about the fact that methylcholanthrene, one of the highly potent carcinogens, has a close chemical relationship to normal constituents of the bile. It has been prepared by drastic chemical methods from desoxycholic acid by Weiland and Dane and from cholic acid by Fieser and Newman. It is true that the areas of the intestine which are most exposed to the bile are the site of far less cancer than is the stomach, which is only mildly exposed. Still there may be a difference of susceptibility of the tissues, and the region of the papilla is the site of most cancers of the duodenum. The several reports of production of tumors by the injection of extracts of bile and liver have recently been reviewed by Steiner. Ghiron and Cook, Kennaway, and Kennaway reported the production of subcutaneous sarcomas in rats and mice by the injection of desoxycholic acid. We tested the carcinogenicity of this substance in mice and rats both by subcutaneous implantation and by inclusion in the diet. Negative results were obtained. This may have been due to differences in the strains of animals used. The lack of concordance might also be explained by the fact that we found that commercial preparations of the acid marketed as "purified" contained as high as 30-percent impurities. Nevertheless the subject still has some attraction.

It has occurred to several authors that the effects of chronic and marked exposure of the stomach to bile might be observed in man in cases where certain surgical procedures had been carried out. With this in mind, we took the opportunity a few years ago to go over the records of a very large hospital. Our most definite conclusion was that, if one excluded all cases in which there was present some factor that materially obscured the interpretation, very few cases would be found for final evaluation, and the solution of the problem by this means would be slow indeed. Out of many thousands of surgical records there were 265 that were related to the problem. All that had not come to secondary operation or to necropsy were, of course, automatically excluded because no definite determination of the condition of the gastric mucosa was possible. Only 6 cases were found in which the pyloroplasty was definitely open and had been so for a considerable period, and in which the mucosa examined for changes was unaffected by the primary lesion. One of the cases had had a cholecystogastrostomy for 4 years in addition to the pyloroplasty. One case was excluded, leaving 5, because no section was taken at secondary operation, and the only description of the appearance of the mucosa by the surgeon was that it was "everywhere intact." Of these 5, all showed gastritic changes; but we could not determine whether the exposure to bile had or had not produced changes in the mucosa. For us the question still remains open.

Ivy has suggested two other possible actions of bile that might be of interest in the induction of gastric cancer. It is possible that the well-known action of bile salts upon fat-soluble substances would influence the action of carcinogenic hydrocarbons in the stomach. Thus the administration of methylcholanthrene in bile might produce a lesion where methylcholanthrene alone failed to do so.

We have seen nothing in the literature with respect to gastric cancer and allergy. That the gastrointestinal tract is subject to acute allergic reactions has been demonstrated. Furthermore in relation to protein allergens, it has been shown by Gray and Walzer that hypoacidity accelerates absorption.

The clinical association of cancer of the stomach with tuberculosis and acanthosis nigricans is mentioned by several authors. One does not at present see a suggestion for investigative work in this, largely because of statistical considerations. It would be very difficult to obtain sufficient cases to settle the question whether the association was real or accidental in the case of the latter, and in the former there are large series of gastric cancers reported with little or no active tuberculosis. However, White reported a series of 300 cases of positive or probable tuberculosis of the stomach in which 10 percent had also a gastric carcinoma.

It has been repeated from time to time in the preceding sections that in many aspects of the problem all or a part of the evidence is based on clinical statistics. In many cases it is evident that available statistics are not complete or adequate. In some of the problems it appears that first clarification may come from extended statistical studies. In discussing gastritis, it was noted that there is some confusion in the use of the term. These facts

indicate the great need for a central agency or registry to handle such matters. In making this statement, the opinions expressed at past gastric cancer conferences are echoed. Such a registry should be more than a pathologic museum and a referee of diagnosis. It should include pathologists, statisticians, epidemiologists, and clerical help. It could serve not only in the collection of data but also in stimulating the collection of better data through educational campaigns and the furnishing of forms and standards. New methods of collecting data, such as have been suggested by Macklin, Warren, Alvarez, and the Bar Harbor conference on parental influence, could be stimulated and coordinated by such a center. The reader will think of other improvements that might be made in this way. It seems reasonable that if such a registry is established and operated along the lines indicated, much new information will be forthcoming. It also seems reasonable to assume that in some cases such new information might save the expenditure of a large amount of time in the laboratory and thus increase progress twofold.

SUMMARY

Gastric cancer ranks very high among causes of death. Its incidence is high and its curability low. This review suggests that a large and important field is open to investigators and that the avenues of approach are many. At present insufficient evidence is available to permit sharp delimitation of the problems, and a tolerant viewpoint regarding the possibilities of various methods of attack has been adopted.

Little is known of heredity in gastric cancer although available evidence indicates that genetic factors play a part. Better data must be collected, particularly in human cases of this disease. It has been suggested that the relationship of gastric cancer to pernicious anemia, achlorhydria, and atrophic gastritis may be indirect and genetically controlled.

The large amount of work that has been done in an attempt to demonstrate a connection between gastric cancer and diet has not resulted in proof of a causal relationship. Many additional experiments are indicated.

No direct evidence of a hormonal influence on gastric cancer has been found. There is much inferential evidence that such an influence may be present, and extensive clinical and animal experimentation is suggested. At present the hormones affecting sex and enterogastrone and related substances appear most interesting.

The experimental production of atrophic gastritis appears to be highly desirable. Its relation to gastric cancer is unknown, but the majority of gastric cancers are associated with atrophic gastritis. Gastritis is also found in association with conditions other than gastric cancer. There is great need for a clarification and standardization of terminology in relation to gastritis. Of methods of producing chronic atrophic gastritis the prolonged use of alcohol has given most encouragement. Hot foods and water and irradiation also appear to be promising agents for this work.

Pernicious anemia appears to have some unknown relationship to gastric cancer. The evidence is entirely clinical and statistical. Efforts to improve or disprove this evidence are needed. Follow-up studies of patients with pernicious anemia have resulted in the finding of early gastric cancer. However, numerical considerations indicate that no large proportion of gastric cancers will be found by such methods.

Achlorhydria appears on statistical evidence to have an important relationship to gastric cancer. The nature of the relationship is obscure. The achlorhydria may occur either before of after the cancer. Gastric analysis is a valuable adjunct to other methods of diagnosis of gastric cancer and should not be omitted. There is a numerical "concentration factor" for gastric carcinoma of unknown nature in the group of people over 45 years of age having achlorhydria. Several experimental studies are suggested.

Proof of the exact relationship of gastric carcinoma to gastric ulcer is lacking. Further experimental evidence on this matter is needed. Many gastric cancers masquerade as ulcers, and improved methods of diagnosis are urgently needed.

Polyps play a definite role in gastric cancer. However, their number is so small in relation to the total of gastric cancers that no great clinical importance can be assigned to this phase of the problem. Both dogs and mice develop spontaneous polyps, and this is a unique advantage in experimentation.

One of the most pressing needs at present is for new or improved methods of diagnosing early gastric cancer. At present the most encouraging innovation is the application of the cytological techniques of Papanicolaou.

The experimental production of gastric adenocarcinoma in animals is still an important problem. The development of the NHO strain of mice which is reported to have spontaneous gastric cancer and the induction of adenocarcinomas of the stomachs of mice by injection of methylcholanthrene are important recent contributions to this field.

The further investigation of mucus and bile in relation to gastric cancer is suggested.

It is suggested in most divisions of this review that available statistics are not so good as one

might desire. The establishment of a gastric cancer registry wherein statistics might be collected and improved would be of great benefit. Much of our present concept of the gastric cancer problem is based upon statistics. If our statistics were improved, certain relations might appear differently than now and much vain effort might be spared.

BIBLIOGRAPHY

GENERAL:

Cancer facilities and services: a report from the National Advisory Cancer Council. Part III. Basic information for use in studying and planning State cancer-control activities, with organization charts as of 1945. J. Nat. Cancer Inst. 6: 249–299 (1946).

Epidemiologic and statistical suggestions. Conference on parental influence in relation to the incidence of human cancer, held at Bar Harbor, Maine, September 21–25, 1944. Published by the Roscoe B. Jackson Memorial Laboratory.

ABRAHAMSON, R. H., and HINTON, J. W.: Carcinoma of the stomach; review of 444 cases to emphasize inadequacy of present methods for early diagnosis. Surg., Gynec. & Obst. 71: 135–141 (1940).

BONNÉ, C.: Cancer in Java and Sumatra. Am. J. Cancer 25: 811–821 (1935).

BOYCE, F. F.: Carcinoma of the stomach in a large general hospital. J. A. M. A. 117: 1670–1675 (1941).

COLLINS, S. D., GOVER, M., and DORN, H. F.: Trend and geographic variation in cancer mortality and prevalence, with special reference to gastric cancer. J. Nat. Cancer Inst. 1: 425–450 (1941).

COX, A. J., JR., and BARNES, V. R.: Experimental hyperplasia of the stomach mucosa. Proc. Soc. Exper. Biol. & Med. 60: 118–120 (1945).

CRILE, G., JR.: Recent developments resulting in an increasing operability rate for cancer of the stomach. Cleveland Clin. Quart. 8: 50–57 (1941).

DOCHAT, G. R., and GRAY, H. K.: Carcinoma of the stomach; prognosis based on a combination of Dukes' and Broders' method of grading. Am. J. Clin. Path. 13: 441–449 (1943).

DORN, H. F.: Illness from cancer in the United States. Pub. Health Rep. 59: 33–48, 65–77, 97–115 (1944).

EWING, J.: Neoplastic diseases; a treatise on tumors. 1160 pp. W. B. Saunders Co., Philadelphia and London (1941).

GOLDSTEIN, H. I.: Cancer of the stomach in children and young adults. Rev. Gastroenterol. 8: 450–458 (1941).

HILL, F. C.: The bearing of gastric secretory mechanism upon the surgical management of gastric and duodenal ulcer. Internat. Abstr. Surg. 73: 133–141 (1941).

HOON, M. R.: Carcinoma of the stomach, a plea for early diagnosis and surgical treatment. Pennsylvania M. J. 46: 929–932 (1942–43).

IVY, A. C.: Digestive system. *In* Cowdry, E. V., Problems of Ageing; Biological and Medical Aspects. Ed. 2, ch. 10. Williams & Wilkins, Baltimore (1942).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

JONES, C. M.: Gastroenterology; a review of the literature from July 1942 to 1943. Arch. Int. Med. 73: 53–108, 154–197 (1944).

KLEIN, A. J., and PALMER, W. L.: Experimental gastric carcinoma; critical review with comments on criteria of induced malignancy. Arch. Path. 29: 814–844 (1940).

LAHEY, F. H.: The bad record of cancer of the stomach. Bull. Lahey Clin. (July 1942).

LINTOTT, G. A. M.: Etiology of cancer of the stomach. I. Factors involved in the varying incidence in different classes and different countries. Guy's Hosp. Rep. 86: 293–300 (1936).

MACCARTY, W. C.: Early cancer of the stomach: a study of 1,299 resected ulcers and 2,408 cancers. Cancer Research 1: 536–537 (1941).

MANN, W. N., SUNDBERG, S., and HERBERT, W. E.: Cancer of the stomach in London, in Stockholm, and in Amsterdam: some comparisons. Guy's Hosp. Rep. 89: 274–284 (1939).

MATEER, J. G., BALTZ, J. L., COMANDURAS, P. D., STEELE, H. H., and BROUWER, S.: Diseases of the stomach: a review of current literature (January 1942–January 1943). Gastroenterology 3: 360–379 (1944).

METHENY, D.: Curability of gastric carcinoma. Northwest Med. 42: 17–18 (1943).

MORRIS, H. J.: End results in surgical treatment of gastric carcinoma. New Orleans M. & Surg. J. 96: 254–262 (1943).

MULLEN, T. F.: Some factors influencing the curability of cancer of the stomach. Surg., Gynec. & Obst. 72: 298–306 (1941).

PACK, G. T., and LIVINGSTON, E. M.: Treatment of cancer and allied diseases. v. 2, pp. 1812, sect. IX, The stomach. Paul B. Hoeber, Inc., New York (1940).

PACK, G. T., and MCNEER, G.: Metastatic cancer of the stomach. Rev. Gastroenterol. 6: 78–84 (1939).

PALMER, W. L., HARMAN, D. S., and RICKETTS, W. E.: Gastroenterology; a review of the literature from July 1943 to June 1944. Arch. Int. Med. 75: 251–278, 341–356, 417–426 (1945).

PALMER, W. L., RICKETTS, W. E., and MAIMON, S. N.: Gastroenterology: a review of the literature from July 1944 to June 1945. Arch. Int. Med. 78: 210–250, 323–366 (1946).

PEACOCK, P. R.: Gastric cancer; an experimental approach to the problem of causation. Glasgow M. J. 139: 117–137 (1943).

PORTIS, S. A., editor: Diseases of the digestive system. Ed. 2. Lea & Febiger, Philadelphia (1944).

SCHINDLER, R.: Gastroscopy; the endoscopic study of gastric pathology. 343 pp. Univ. Chicago Press, Chicago (1937).

STOUT, A. P.: Superficial spreading type of carcinoma of the stomach. J. Nat. Cancer Inst., 5: 363 (1945).

VOEGTLIN, C.: A program for the study of cancer of the stomach. J. Nat. Cancer Inst. 1: 539–543 (1941).

WALTERS, W., GRAY, H. K., and PRIESTLEY, J. T.: Carcinoma and other malignant lesions of the stomach. 576 pp. W. B. Saunders Co., Philadelphia (1942).

WHITE, R. R.: Simultaneous carcinoma and tuberculosis of the stomach in a case of pernicious anemia. Proc. Staff Meet., Mayo Clin. 18: 165–172 (1943).

HEREDITY:

Epidemiologic and statistical suggestions. Conference on parental influence in relation to the incidence of human cancer, at Bar Harbor, Maine, September 21–25, 1944. Published by the Roscoe B. Jackson Memorial Laboratory.

ANDERVONT, H. B.: Development and genetic characteristics of the adenomatous stomach lesion in strain I mice. Pub. Health Rep. 54: 1851–1855 (1939).

ANDERVONT, H. B., and STEWART, H. L.: Adenomatous lesion in the stomach of strain I mice. Science 86: 566–567 (1937).

BAUER, J.: The relation between peptic ulcer and cancer of the stomach from the genetic point of view. Rev. Gastroenterol. 7: 21–24 (1940).

BLANK, F.: Genetic aspects of the cancer problem; preliminary report on survey of constitution as related to cancer. Arch. Surg. 49: 301–315 (1944).

BONNÉ, C., HARTZ, P. H., KLERKS, J. V., POSTHUMA, J. H., RADSMA, W., and TJOKRONEGORO, S.: Morphology of the stomach and gastric secretion in Malays and Chinese and different incidence of gastric ulcer and cancer in these races. Am. J. Cancer 33: 265–279 (1938).

HABS, H.: Krebs und Vererbung. Ztschr. f. klin. Med. 135: 676–691 (1938–39).

HABS, H.: Zwillingsforschung und Krebskrankheit unter besonderer Berücksichtigung einer auslesefreien Zwillingsserie von 100 tumorkranker Zwillingspaaren. Deutsche med. Wchnschr. 65: 1766–1769 (1939).

HAUSER, I. J., and WELLER, C. V.: A further report on the cancer family of Warthin. Am. J. Cancer 27: 434–449 (1936).

HURST, A. F.: Cancer of the alimentary tract; its pathogenesis and prophylaxis. Lancet 1: 553–558, 621–626 (1939).

IVY, A. C., and GROSSMAN, M. I.: The genetic factor in gastric cancer. (Comment). Gastroenterology 6: 65–66 (1946).

LEVIN, A. E., and KUCHUR, B. A.: Investigation of relatives of patients with gastric cancer. Lancet 1: 204–205 (1937).

MACKLIN, M. T.: An analysis of tumors in monozygotic and dizygotic twins. J. Hered. 31: 270–290 (1940).

SOKOLOFF, B.: Predisposition to cancer in the Bonaparte family. Am. J. Surg. 40: 673–678 (1938).

STRONG, L. C.: Genetic analysis of induction of tumors by methylcholanthrene. IX. Induced and spontaneous adenocarcinomas of the stomach in mice. J. Nat. Cancer Inst. 5: 339–362 (1945).

STRONG, L. C., COLLINS, V. J., and DURAND, E. A.: Genetic analysis of induction of tumors by methylcholanthrene. IV. Probably remote induction of various types of gastric lesions. Cancer Research 3: 21–28 (1943).

VON VERSCHUER, O., and KOEER, E.: Die Frage der erbicken Disposition zum Krebs. Vorläufige Mitteilung über eine auslesefreie Zwillingsserie. Ztschr. f. Krebsforsch. 50: 5–14 (1940).

DIET AND VITAMINS:

ABELS, J. C., ARIEL, I., REKER, P. E., PACK, G. T., and RHOADS, C. P.: Metabolic abnormalities in patients with cancer of gastrointestinal tract; review of recent studies. Arch. Surg. 46: 844–860 (1943).

ABELS, J. C., GORHAM, A. T., PACK, G. T., and RHOADS, C. P.: Metabolic study in patients with cancer of the gastro-intestinal tract. I. Plasma vitamin A levels in patients with malignant neoplastic disease, particularly of the gastro-intestinal tract. J. Clin. Investigation 20: 749–764 (1941).

ABELS, J. C., GORHAM, A. T., PACK, G. T., and RHOADS, C. P.: Metabolic studies in patients with gastro-intestinal cancer. III. Hepatic concentrations of vitamin A. Proc. Soc. Exper. Biol. & Med. 48: 488–492 (1941).

BECK, S., and PEACOCK, P. R.: Gastro-papillomatosis due to vitamin A deficiency induced by heated fats. Brit. M. J. 2: 81–83 (1941).

BERG, B. N.: Pathological changes in nutritional gastritis in rats. Am. J. Path. 18: 49–61 (1942).

BRUNSCHWIG, A., and RASMUSSEN, R. A.: The relation of diet to benign neoplasia (ulcero-papillomas) of the rat's stomach. Cancer Research 1: 371–378 (1941).

BURK, D., and WINZLER, R. J.: Vitamins and cancer. *In* Vitamins and Hormones, v. 2. (1944).

CHESLEY, F. F., DUNBAR, J., and CRANDALL, L. A.: The vitamin B complex and its constituents in functional digestive disturbances. Am. J. Digest. Dis. 7: 24–27 (1940).

COWGILL, G. R., and GILMAN, A.: Physiology of vitamins. XXIII. The effect of lack of the vitamin B complex on the secretion of gastric juice in dogs with gastric pouches. Arch. Int. Med. 53: 58–70 (1934).

CRAMER, W.: The prevention of cancer. Lancet 1: 1–5 (1934).

DICKENS, F., and WEIL-MALHERBE, H.: Investigation into the possible carcinogenic activity of wood smoke. Cancer Research 2: 680–684 (1942).

DOMAGK, G.: Weitere experimentelle Untersuchungen über die Ursachen des Krebses. Ztschr. f. Krebsforsch. 48: 283–297 (1939).

DUNHAM, L. J., and BRUNSCHWIG, A.: A review of dietary and related habits in patients with malignant gastric neoplasms. Gastroenterology 6: 286–293 (1946).

FUJIMAKI, Y.: Formation of gastric carcinoma in albino rats fed on deficient diets. J. Cancer Research 10: 469–477 (1926).

GOLDBLATT, H., and BENISCHEKS, M.: Vitamin A deficiency and metaplasia. J. Exper. Med. 46: 699–707 (1927).

GROSSMAN, M. I., and IVY, A. C.: Diet and cancer. Comment. Gastroenterology 4: 186–187 (1945).

HERBERT, W. E., and BRUSKE, J. S.: Etiology of cancer of the stomach. II. A comparison of the diet and dental condition of the English and Dutch, with special reference to gastric irritants. Guy's Hosp. Rep. 86: 301–308 (1936).

IACOBOVICI, I., BALTACEANU, G., COMANESCO, A., and VASILESCO, G. E. M.: Researches on the vitamin C content of the gastric wall in different affections of the stomach. Bull. Acad. de méd. de Roumanie 17: 187–194 (1945).

KIRBY, A. H. M.: Attempts to induce stomach tumors. I. The effect of cholesterol heated to 300° C. Cancer Research 3: 519–525 (1943).

KIRBY, A. H. M.: Attempts to induce stomach tumors. III. The effects of (a) a residue of cholesterol heated to 300° C., and (b) 3,5-cholestadiene. Cancer Research 4: 94–97 (1944).

KIRBY, A. H. M.: Attempts to induce stomach tumors. IV. The effect of (a) cholesteryl esters heated to 300° C., and (b) cholesterol heated to 430° C. Cancer Research 5: 129–134 (1945).

KLEIN, A. J., and PALMER, W. L.: Experimental gastric carcinoma; critical review with comments on criteria of induced malignancy. Arch. Path. 29: 814–844 (1940).

LARSEN, C. D., and BARRETT, M. K.: Administration of 3, 5-cholestadiene and dicholesteryl ether to mice and rats. J. Nat. Cancer Inst. 4: 587–594 (1944).

LAYNE, J. A., and CAREY, J. B.: The effect of a black-tongue-producing diet upon the endoscopic appearance of the gastric mucosa in the dog. Gastroenterology 2: 133–141 (1944).

LORENZ, E., and STEWART, H. L.: Intestinal carcinoma and other lesions in mice following oral administration of 1, 2, 5, 6-dibenzanthracene and 20-methylcholanthrene. J. Nat. Cancer Inst. 1: 17–40 (1940).

MANN, W. N., SUNDBERG, S., and HERBERT, W. E.: Cancer of the stomach in London, in Stockholm, and in Amsterdam; some comparisons. Guy's Hosp. Rep. 89: 274–284 (1939).

MORRIS, H. P., LARSEN, C. D., and LIPPINCOTT, S. W.: Effects of feeding heated lard to rats, with a histologic description of the lesions observed. J. Nat. Cancer Inst. 4: 285–303 (1943).

ROFFO, A. H.: Tumeurs malignes developpées dans l'appareil digestif par l'ingestion de graisses oxydées par chauffage. Bull. Assoc. franç. p. l'étude du cancer 28: 556–588 (1939).

ROFFO, A. H.: Cancerización gástrica por ingestion de alquitrán tabáquico. Prensa méd. argent. 29: 18–35 (1942).

RUSCH, H. P.: Extrinsic factors that influence carcinogenesis. Physiol. Rev. 24: 177–204 (1944).

SCHABAD, L. M.: Action of coal-tar administered per os and per rectum in white mice. Vestnik Roentgenol. i. Radiol., Leningrad 4: 166–179 (1926).

SHAPIRO, N., SCHIFF, L., BLOCH, H. S., GARBER, E. S., and HANNAHER, M. J.: Some observations on specific vitamins and atrophic gastritis. Gastroenterology 2: 121–132 (1944).

SHARPLESS, G. R.: Neoplastic hyperplasia of mucosa in rats. Proc. Soc. Exper. Biol. & Med. 34: 684–685 (1936).

STREET, H. R., COWGILL, G. R., and ZIMMERMAN, H. M.: Some observations of vitamin B6 deficiency in the dog. J. Nutrition 21: 275–280 (1941).

SUGIURA, K.: The relation of diet to the development of gastric lesions in the rat. Cancer Research 2: 770–775 (1942).

WATERMAN, N.: Cancer expérimentale de l'estomac: son rapport avec la genèse du cancer de l'estomac humain. Bull. Assoc. franç. p. l'étude du cancer 29: 76–79 (1940).

HORMONES:

ABRAHAMSON, R. H., CHURCH, R., and HINTON, J. W.: Hormone effect on the male gastroduodenal mucosa. Am. J. M. Sc. 204: 809–823 (1942).

ABRAHAMSON, R. H., and HINTON, J. W.: The gastric mucosa as an endocrine gland. Surg., Gynec. & Obst. 76: 147–163 (1943).

ATKINSON, A. J., and IVY, A. C.: Further attempts to produce achlorhydria. Am. J. Digest. Dis. 5: 30–35 (1938).

BERRYHILL, W. R., and WILLIAMS, H. A.: A study of the gastric secretion in hyperthyroidism before and after operation. J. Clin. Investigation 11: 753–760 (1932).

BROAD, G. G., and BERMAN, L. G.: Treatment of experimental Mann-Williamson ulcers with anterior pituitary-like hormone (antuitrin-S). Am. J. Digest. Dis. 8: 27–28 (1941).

BRUNSCHWIG, A., VAN PROHASKA, J., CLARKE, T. H., and KANDEL, E. V.: Secretory depressant in the gastric juice of patients with pernicious anemia. J. Clin. Investigation 18: 415–422 (1939).

DODDS, E. C., NOBLE, R. L., SCARFF, R. W., and WILLIAMS, P. C.: Pituitary control of alimentary blood flow and secretion; changes in the stomach produced by administration of posterior pituitary extract. Proc. Roy. Soc., London, s. B, 123: 22–26 (1937).

GRAY, J. S.: Present status of urogastrone. Am. J. Digest. Dis. 8: 365–366 (1941).

GROSSMAN, M. I., GREENGARD, H., DUTTON, D. F., and WOOLLEY, J. R.: The effect of prolonged administration of enterogastrone on gastric secretion in normal and Mann-Williamson dogs. Gastroenterology 2: 437–441 (1944).

HANDS, A. P., GREENGARD, H., FAULEY, G. B., and IVY, A. C.: Prevention of experimental gastrojejunal ulcer by enterogastrone. Fed. Am. Soc. Exper. Biol. 2: 18 (1943).

GÜLZOW, M.: Ulkus- und Gastritisbehandlung mit Sexualhormonen. Deutsche med. Wchnschr. 68: 1071–1074 (1942).

HOLLANDER, F.: Gastric hypersecretion following parturition in a dog. Proc. Soc. Exper. Biol. & Med. 27: 303–304 (1930).

IVY, A. C.: Some recent developments in the physiology of the stomach which pertain to the management of peptic ulcer. Bull. New York Acad. Med. 20: 5–14 (1944).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

MCNEER, G.: Cancer of the stomach in the young. Am. J. Roentgenol. 45: 537–550 (1941).

MAGRUDER, S. R.: Unusual reactions of mucosa following hypophysectomy. Anat. Rec. 73: 269–271 (1939).

METZ, M. H., and LACKEY, R. W.: The treatment of peptic ulcer with posterior pituitary extracts. Texas State J. Med. 32: 589–590 (1937).

NASIO, J.: Action of stilbestrol by parenteral route on development of peptic ulcer produced experimentally with cinchophen. Prensa méd. argent. 33: 859–862 (1946).

NASIO, J.: Action of stilbestrol in the prevention of cinchophen peptic ulcer in castrated dogs. Prensa méd. argent. 33: 1693–1695 (1946).

NECHELES, T.: Enterogastrone, urogastrone, and anthelone. (Editorial). Gastroenterology 5: 427–429 (1945).

OLIVER, T. H., and WILKINSON, J. F.: Achlorhydria. Quart. J. Med. 2: 431–462 (1933).

QUIGLEY, J. P.: Enterogastrone—significant steps in development of the present conception. Am. J. Digest. Dis. 8: 363–364 (1941).

RASMUSSEN, R. A., and BRUNSCHWIG, A.: Peptic activity of achlorhydric human gastric juices from carcinomatous stomachs. Comparative study. Proc. Soc. Exper. Biol. & Med. 46: 289–300 (1941).

SANDWEISS, D. J.: Enterogastrone, anthelone, and urogastrone; a review of the literature and a suggestion for the organization of a national committee for the study of "peptic" ulcer. Gastroenterology 5: 404–415 (1945).

SCHIFFRIN, M. J., and GRAY, J. S.: The effect of urogastrone on gastric secretion in enterectomized dogs. Am. J. Physiol. 137: 417–420 (1942).

SCHULZ, W.: Die Behandlung der Ulzerationen am Magen-Darmtraktus mit weiblichem Sexualhormon. Med. Klin. 35: 914–917 (1939).

SCOTT, V. B., MOE, R., and BRUNSCHWIG, A.: Further studies on properties of gastric secretory depressant in gastric juice. Proc. Soc. Exper. Biol. & Med. 52: 45–46 (1943).

SHAY, H., GERSHON-COHEN, J., and FELS, S. S.: Gastric anacidity: its physiologic and clinical significance and its management. Am. J. Digest. Dis. 8: 115–123 (1941).

STRAUSS, M. B., and CASTLE, W. B.: Studies of anemia in pregnancy: etiologic relationship of gastric secretory defects and dietary deficiency to hypochromic and macrocytic (pernicious) anemias of pregnancy and the treatment of these conditions. Am. J. M. Sc. 185: 539–551 (1933).

TUERKISCHER, E., and WERTHEIMER, E.: Adrenalectomy and gastric secretion. J. Endocrinol. 4: 143–151 (1945).

WAY, S.: Relation between gastric acidity and the anterior pituitary-like hormone content of urine in pregnant women. Brit. M. J. 2: 182–184 (1945).

WIECZOROWSKI, E., GRAY, J. S., and IVY, A. C.: The effect of urogastrone on the gastric secretion in man. Am. J. Physiol. 129: 496 (1940).

BACTERIOLOGY:

APPELMANS, R., and VASSILIADIS, P.: Étude sur la flore microbienne des ulcères gastro-duodénaux et des cancers gastriques. Rev. belge sc. méd. 4: 198–203 (1932).

BARBER, M., and FRANKLIN, R. R.: Bacteriology of stomach and duodenum in cases of peptic ulcer and gastric carcinoma. Brit. M. J. 1: 951–953 (1946).

BONNÉ, C., and SANDGROUND, J. H.: On the production of gastric tumors bordering on malignancy, in Javanese monkeys through the agency of Nochtia nochtia, a parasitic nematode. Am. J. Cancer 37: 173–185 (1939).

LAMPE, A. E., and STRASSBURGER, H.: Bacteriology of the healthy and the diseased stomach. Arch. f. Hyg. 130: 150–180 (1943).

PRIESTLEY, J. T., THOMPSON, L., and SEALY, W. B.: Bacteriologic aspects of gastric contents in presence of surgical lesions of the stomach and duodenum. Proc. Staff Meet., Mayo Clin. 19: 1–4 (1944).

SAUNDERS, E. W.: Is there a specific bacterial irritant to four sites of carcinoma? Am. J. Cancer 15: 2745–2755 (1931).

SELEY, G. P. and COLP, R.: The bacteriology of peptic ulcer and gastric malignancies; possible bearing on complications following gastric surgery. Surgery 10: 369–380 (1941).

ATROPHIC GASTRITIS:

AFENDULIS, T., and GÜLZOW, M.: Die allergisch-hyperergische Gastritis. Tierexperimentelle Untersuchungen. Ztschr. f. d. ges. exper. Med. 104: 167–181 (1938).

ALVAREZ, W. C.: Cancer of the stomach arising in gastritis. Proc. Staff Meet., Mayo Clin. 18: 225–226 (1943).

BAKER, C. P.: Histopathology of chronic gastritis; preliminary report. Proc. Staff Meet., Mayo Clin. 11: 521–522 (1936).

BEAZELL, J. M., and IVY, A. C.: The influence of alcohol on the digestive tract; review. Quart. J. Studies on Alcohol 1: 45–73 (1940).

BENNETT, T. I.: Discussion on gastritis. Proc. Roy. Soc. Med. 38: 81–82 (1944).

BERG, B. N.: Pathological changes in nutritional gastritis in rats. Am. J. Path. 18: 49–61 (1942).

BERRY, L. H.: Chronic alcoholic gastritis; evaluation of concept, with gastroscopic studies in 100 cases. J. A. M. A. 117: 2233–2236 (1941).

BONNÉ, C.: Cancer in Java and Sumatra. Am. J. Cancer 25: 811–821 (1935).

BRINDLEY, G. V., JR., DOCKERTY, M. B., and GRAY, H. K.: Multiple carcinomas of the stomach; report of cases. Proc. Staff Meet., Mayo Clin. 18: 193–197 (1943).

CAREY, J. B.: Gastroscopic observations in chronic gastritis. Am. J. Digest. Dis. 7: 160–164 (1940).

COVARRUBIAS ZENTENO, R.: Gastritis quirurgica experimental. Rev. méd. de Chile 72: 884–887 (1944).

CUNHA, F.: Chronic gastritis. Rev. Gastroenterol. 11: 239–245 (1944).

DAWSON, A. B.: Histologic changes in the gastric mucosa (Pavlov pouch) of a dog following irradiation. Am. J. Roentgenol. & Radium Therap. 13: 320–326 (1925).

EBSTEIN, W.: Ueber die Veränderungen, welche die Magenschleimhaut durch die Einverleibung von Alkohol und Phosphor in den Magen erleidet. Virchows Arch. f. path. Anat. 55: 469 (1872).

FABER, K.: Chronic gastritis; its relation to achylia and ulcer. Lancet 213: 901–907 (1927).

FABER, K.: Gastritis and its consequences. 119 pp. Oxford Univ. Press, London (1935).

FENWICK, S.: On atrophy of the stomach. Lancet 2: 78–80 (1870).

FENWICK, S.: Lecture on atrophy of the stomach. Lancet 2: 1–4 (1877).

GOLDING, F. C.: The association of atrophic gastritis with hypothyroidism; a preliminary report of 11 cases. Ann. Int. Med. 17: 828–834 (1942).

GOODPASTURE, E. W.: An anatomical study of senescence in dogs, with especial reference to the relation of cellular changes of age to tumors. J. M. Research 38: 127–189 (1918).

GRAY, S., and SCHINDLER, R.: Gastric mucosa of chronic alcoholic addicts; gastroscopic study. J. A. M. A. 117: 1005–1011 (1941).

GUISS, L. W., and STEWART, F. W.: Chronic atrophic gastritis and cancer of the stomach. Arch. Surg. 46: 823–843 (1943).

GUTMANN, R. A., BERTRAND, I., and PÉRISTIANY, T. J.: Le cancer de l'estomac au début: étude clinique, radiologique et anatome-pathologique. G. Doin & Cie., Paris (1939).

HARDT, L. I., HUFFORD, A. R., and RABENS, J. I.: Analytical survey of 1132 patients gastroscopically examined. Gasteroenterology 4: 477–483 (1945).

HARING, W.: Welche Bedeutung besitzt die chronische Gastritis für die Entstehung des Magenkrebses. Med. Klin. 35: 1284–1286 (1939).

HEBBEL, R.: Chronic gastritis; its relation to gastric and duodenal ulcer and to gastric carcinoma. Am. J. Path. 19: 43–71 (1943).

HILLENBRAND, K.: Histotopographische und histologische Untersuchungen über die sogenannten chronische Gastritis. Beitr. z. path. Anat. u. z. allg. Path. 85: 1–32 (1930).

HURST, A. F.: Schorstein lecture on precursors of carcinoma of the stomach. Lancet 2: 1023–1028 (1929).

HURST, A. F.: The clinical importance of achlorhydria. Brit. M. J. 2: 665–669 (1934).

IVY, A. C., ORNDOFF, B. H., JACOBY, A., and WHITLOW, J. E.: Studies on the effect of X-ray on glandular activity. III. The effect of X-ray on gastric secretion. Radiology 1: 39–46 (1923).

JANKELSON, I. R., MCCLURE, C. W., and FREEDBERG, H.: Relation of atrophic gastric mucosa to carcinoma of the stomach. Rev. Gastroenterol. 10: 26–31 (1943).

JONES, C. M.: Clinical evaluation of gastritis. Am. J. Digest. Dis. 8: 205–209 (1941).

JONES, F. A.: Discussion on gastritis. Proc. Roy. Soc. Med. 38: 82–84 (1944).

JUDD, E. S., JR.: Possible relationship of residual lesions of ulcerative gastritis to the development of carcinomas of the stomach. Proc. Staff Meet., Mayo Clin. 14: 52–56 (1939).

JUDD, E. S., JR.: Residual lesions of ulcerative gastritis; possible relationship to carcinoma of the stomach. Surg., Gynec. & Obst. 75: 424–432 (1942).

KENAMORE, B., SCHEFF, H., and WOMACK, N. A.: Study of gastric lesions by means of biopsy specimens removed endoscopically. Arch. Surg. 52: 50–58 (1946).

KONJETZNY, G. E.: Ueber die Beziehungen der chronischen Gastritis mit ihren Folgeerscheinungen und des chronischen Magenulcus zur Entwicklung des Magenkrebses. Beitr. z. klin. Chir. 85: 455–519 (1913).

KONJETZNY, G. E.: Chronische Gastritis und Magenkrebs. Monatsschr. f. Krebsbekämpf. 2: 65–78 (1934).

KONJETZNY, G. E.: Relation between gastritis and the development of stomach cancer. Arch. f. klin. Chir. 204: 4–64 (1942–43).

LESER, R. U., and HASTERLIK, R. J.: A case of atrophic gastritis successfully treated with liver-stomach concentrate. Am. J. Digest. Dis. 9: 78–79 (1942).

MAGNUS, H. A.: Observations on the presence of intestinal epithelium in the gastric mucosa. J. Path. & Bact. 44: 389–398 (1937).

MAHER, M. M., ZINNINGER, M. M., SCHIFF, L., and SHAPIRO, N.: Some observations on gastritis and peptic ulcer. Am. J. M. Sc. 205: 328–333 (1943).

MEISSNER, W. A.: Observations on chronic gastritis and cancer. J. Nat. Cancer Inst. 5: 377 (1945).

MORLEY, J.: Carcinoma of the stomach. Brit. M. J. 2: 949–953 (1937).

NEPVEU, P. F.: Sur le cancer de l'estomac, considéré comme l'une des terminaisons de la gastrite chronique. v. 167, No. 139, 24 pp. Paris (1821).

ORATOR, V.: Beiträge zur Magenpathologie. I. Das Magen-Duodenal und postoperative Jejunal geschwür. Virchows Arch. f. path. Anat. 255: 639–676 (1925).

ORATOR, V.: Beiträge zur Magenpathologie. II. Zur Pathologie und Genese des Carcinoms und Ulcuscarcinoms des Magens. Virchows Arch. f. path. Anat. 256: 202–229 (1925).

ORATOR, V.: Beiträge zur Magenpathologie. III. Nicht carcinomatöse Tumoren und Entzündungen des Magens, studiert an chirurgischem Resektionsmaterial. Virchows Arch. f. path. Anat. 256: 230–242 (1925).

ORTMAYER, M., BALKIN, R., and HUMPHREYS, E.: Chronic erosive, granulomatous, atrophic gastritis. Gastroenterology 6: 298–301 (1946).

OSTROUCH, M.: Action de l'alcool éthylique sur les structures cytoplasmiques les cellules de l'epithelium superficial de l'estomac. Arch. de biol. 43: 321–341 (1932).

PALMER, W. L., and TEMPLETON, F. E.: Effect of radiation therapy on gastric secretion. J. A. M. A. 112: 1429–1434 (1939).

SCHINDLER, R.: Gastroscopy: the endoscopic study of gastric pathology. Univ. Chicago Press, Chicago (1937).

SCHINDLER, R.: Chronic gastritis. Bull. New York Acad. Med. 15: 322–337 (1939).

SCHINDLER, R.: Benign tumors. *In* Portis, S. A., editor, Diseases of the Digestive System. Ed. 2. Lea & Febiger, Philadelphia (1944).

SCHINDLER, R., NECHELES, H., and GOLD, R. L.: Surgical gastritis; study on genesis of gastritis found in resected stomachs with particular reference to so called "antral gastritis" associated with ulcer. Surg., Gynec. & Obst. 69: 281–286 (1939).

SCHINDLER, R., and ORTMAYER, M.: Histopathology of chronic gastritis. Am. J. Digest. Dis. 9: 411–415 (1942).

SCHINDLER, R., and SERBY, A. M.: Gastroscopic observations in pernicious anemia. Arch. Int. Med. 63: 334–355 (1939).

SHAPIRO, N., SCHIFF, L., BLOCH, H. S., GAEBER, E. S., and HANNAHER, M. J.: Some observations on specific vitamins and atrophic gastritis. Gastroenterology 2: 121–132 (1944).

SHAPIRO, N., SCHIFF, L., MAHER, M. M., and ZINNINGER, M. M.: Some observations on atrophic gastritis and gastric cancer. J. Nat. Cancer Inst. 2: 583–588 (1942).

SMITH, G. M.: Morphological changes in tissues with changes in environment. III. Gastric mucous membrane implanted into the intestine. J. M. Research 33: 337–343 (1916).

SNELL, A. M., and BOLLMAN, J. L.: Gastric secretion following irradiation of the exposed stomach and upper abdominal viscera by roentgen rays. Am. J. Digest. Dis. & Nutrition 1: 164–168 (1934).

STALKER, L. K., BOLLMAN, J. L., and MANN, F. C.: Effect of cinchophen on the gastric secretion: an experimental study. Arch. Surg. 34: 1172–1178 (1937).

STOUT, A. P.: Pathology of carcinoma of the stomach. Arch. Surg. 46: 807–822 (1943).

STOUT, A. P.: Gastric mucosal atrophy and carcinoma of the stomach. New York State J. Med. 45: 973–977 (1945).

THOMSEN, E.: Études sur l'achylie neurogène et cellulaire. Acta. med. scandinav. 61: 377–432 (1925).

THOMSEN, E.: Études sur l'achylie neurogène et cellulaire. II. Influence de la cholécystectomie sur la sécrétion gastrique du chien. Acta. med. scandinav. 61: 522–569 (1924–25).

TUMEN, H. J., and LIEBERTHAL, M. M.: Chronic gastritis; a review of its present status. Internat. Clin. 2: 263–291 (1941).

USLAND, O.: Ueber die Bedeutung der chronischen Gastritis für die Entwicklung des Magenkrebses. Acta. chir. scandinav. 76: 485–499 (1935).

VELDE, G.: Die Beziehungen zwischen perniziöser Anämie, Magenpolypen und Magencarcarcinom. Ztschr. f. klin. Med. 134: 653–670 (1938).

WANSER, R.: Die banale chronische Gastritis und ihre Beziehungen zum Magenkarzinom. Beitr. z. path. Anat. u. z. allg. Path. 103: 113–156 (1939).

WARREN S., and MEISSNER, W. A.: Chronic gastritis and carcinoma of the stomach. Gastroenterology 3: 251–276 (1944).

WESTHUES, H.: Entstehung des Magencarcinoms aus Gastritis und entzundlichen Polypen? Arch. f. klin. Chir. 203: 391–435 (1942).

PERNICIOUS ANEMIA:

Gastric cancer and pernicious anemia. (Editorial comment). Lancet 2: 406–407 (1945).

ALSTED, G.: On free hydrochloric acid in stomach in pernicious anemia. Acta med. scandinav. 82: 288–299 (1934).

ASKEY, J. M.: Addisonian pernicious anemia without achlorhydria; does it exist? Gastroenterology 2: 1–12 (1944).

BROWN, M. R.: The pathology of the gastro-intestinal tract in pernicious anemia and subacute combined degeneration of the spinal cord; study of 151 autopsies. New England J. Med. 210: 473–477 (1934).

BRUNSCHWIG, A., VAN PROHASKA, J., CLARKE, T. H., and KANDEL, E. V.: Secretory depressant in the gastric juice of patients with pernicious anemia. J. Clin. Investigation 18: 415–422 (1939).

BUSSABARGER, R. A., and IVY, A. C.: Hematologic studies on gastrectomized monkeys. Proc. Soc. Exper. Biol. & Med. 34: 151–152 (1936).

BUSSABARGER, R. A., IVY, A. C., WIGODSKY, H. S., and GUNN, F. D.: The effect of gastrectomy on the monkey. Ann. Int. Med. 13: 1028–1041 (1939).

BUSSABARGER, R. A., and JUNG, F. T.: Dietary and hematologic studies after gastrectomy in the rat. Am. J. Physiol. 117: 59–66 (1936).

CAREY, J. B.: Gastroscopic observations in chronic gastritis. Am. J. Digest. Dis. 7: 160–164 (1940).

CAREY, J. B.: Gastroscopic observations in pernicious anemia. Minnesota Med. 23: 311–313 (1940).

CASTLE, W. B., HEATH, C. W., and STRAUSS, M. B.: Observations on etiologic relationship of achylia gastrica to pernicious anemia. IV. Biologic assay of gastric secretion. . . Am. J. M. Sc. 182: 741–764 (1931).

CONNER, H. M., and BIRKELAND, I. W.: Coexistence of pernicious anemia and lesions of the gastrointestinal tract; carcinoma of the stomach; consideration of 20 cases; 11 reported. Ann. Int. Med. 7: 89–104 (1933).

COTTI, L.: Rilievi casistico- bibliografici e contributo personale in merito alla conoscenza dei rapporti fra anemia perniciosa e carcinoma gastrico. Haematologica 19: 939–982 (1938).

COX, A. J.: The stomach in pernicious anemia. Am. J. Path. 19: 491–501 (1943).

DENNIG, H.: Perniziöse Anämie nach Magensresektion. München. med. Wchnschr. 76: 633 (1929).

DOEHRING, P. C., and EUSTERMAN, G. B.: Association of pernicious anemia and carcinoma of the stomach. Arch. Surg. 45: 554–563 (1942).

DYKE, S. C., and HARVEY, E.: The efficient treatment of pernicious anemia. Lancet 2: 59–62 (1933).

FARRIS, J. M., RANSOM, H. K., COLLER, F. A.: Total gastrectomy; effect upon nutrition and hematopoiesis. Surgery 13: 823–833 (1943).

FENWICK, S.: On atrophy of the stomach. Lancet 2: 78–80 (1870).

FENWICK, S.: Lecture on atrophy of the stomach. Lancet 2: 1–4 (1877).

GOLDHAMER, S. M.: Macrocytic anemia in cancer of stomach, apparently due to lack of intrinsic factor. Am. J. M. Sc. 195: 17–10 (1938).

GRIFFIN, H. Z., and BOWLER, J. P.: Diseases which may be associated with pernicious anemia. Minnesota Med. 6: 13–16 (1923).

HELD, I. W., and GOLDBLOOM, A. A.: Cancer of the stomach in a cured case of Addison-Biermer's (pernicious) anemia. J. A. M. A. 108: 1398–1400 (1937).

HURST, A. F.: Achlorhydria and achylia, and their connexion with the Addison's anemia-subacute combined degeneration syndrome and simple (non-Addisonian) achlorhydric anemia. Quart. J. Med. 1: 157–177 (1932).

IVY, A. C.: The effects of gastrectomy in animals. Am. J. Digest. Dis. 7: 500–502 (1940).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

JANKELSON, I. R., MCCLURE, C. W., and FREEDBERG, H.: Relation of atrophic gastric mucosa to carcinoma of the stomach. Rev. Gastroenterol. 10: 26–31 (1943).

JENNER, A. W. F.: Perniziöse Anämie und Magenkarzinom. Acta med. scandinav. 102: 529–590 (1939).

JOHANSEN, A. H.: Achylia in pernicious anemia after liver treatment; examination after histamine injection. J. A. M. A. 92: 1728–1730 (1929).

JONES, C. M.: Clinical evaluation of gastritis. Am. J. Digest. Dis. 8: 205–209 (1941).

JONES, C. M., BENEDICT, E. B., and HAMPTON, A. O.: Variations in the gastric mucosa in pernicious anemia; gastroscopic, surgical and roentgenologic observations. Am. J. M. Sc. 190: 596–610 (1935).

KAPLAN, H. S., and RIGLER, L. G.: Pernicious anemia and carcinoma of the stomach—autopsy studies concerning their interrelationship. Am. J. M. Sc. 209: 339–348 (1945).

LAHEY, F. H., and MARSHALL, S. F.: Indications for, and experiences with total gastrectomy based upon 73 cases of total gastrectomy. Ann. Surg. 119: 300–320 (1944).

LESER, R. U., and HASTERLIK, R. J.: A case of atrophic gastritis successfully treated with liver-stomach concentrate. Am. J. Digest Dis. 9: 78–79 (1942).

McI'EAK, E. M., and NEIGHBORS, DE W.: Minot-Murphy diet in pernicious anemia; report of 5 cases. South. M. J. 20: 926–931 (1927).

MAGNUS, H. A., and UNGLEY, C. C.: The gastric lesion in pernicious anemia. Lancet 1: 420–421 (1938).

MAZZINI, O. F., and SALVI, M.: Modificaciones del quimismo gástrico y de la fórmula sanguínea postgastrectomía. Prensa méd. argent. 32: 375–381 (1945).

MEULENGRACHT, E.: Histologic investigation into pyloric gland organ in pernicious anemia. Am. J. M. Sc. 197: 201–214 (1939).

MILLER, T. G.: The relation of gastritis to the anemias. Internat. Clin. 1: 173–178 (1940).

OLSON, S. W., and HECK, F. J.: Lesions of the gastric mucosa in pernicious anemia. Proc. Staff Meet., Mayo Clin. 20: 74–78 (1945).

OPPENHEIM, A., ABELS, J. C., PACK, G. T., and RHOADS, C. P.: Metabolic studies in patients with cancer of the gastro-intestinal tract. XIX. The anemia of patients with gastric carcinoma. J. A. M. A. 127: 273–276 (1945).

PORGES, O.: Magenkrankheiten, ihre Diagnose und Therapie. pp. 163–164. Urban & Schwarzenberg, Berlin (1929).

RHOADS, C. P.: Gastric anacidity as a sequel to gastritis, particularly the gastritis of pernicious anemia. J. Nat. Cancer Inst. 1: 511–522 (1941).

ROZENDAAL, H. M., and WASHBURN, R. N.: Gastric secretion in cases of pernicious anemia. Ann. Int. Med. 11: 1834–1837 (1938).

RIGLER, L. R., KAPLAN, H. S., and FINK, D. L. Pernicious anemia and the early diagnosis of tumors of the stomach. J. A. M. A. 128: 426–432 (1945).

SCHENKEN, J. R., STASNEY, J., and HALL, W. K.: The antianemic principle in the human liver in carcinomas of the stomach and cecum. Am. J. M. Sc. 200: 11–17 (1940).

SCHINDLER, R., and SERBY, A. M.: Gastroscopic observations in pernicious anemia. Arch. Int. Med. 63: 334–355 (1939).

SCHLICKE, C. P.: The effect of normal and abnormal human gastric juice, administered to the mother, on the blood of newborn rats. Preliminary report. Proc. Staff Meet., Mayo Clin. 14: 145–149 (1939).

SCHLICKE, C. P.: Studies on the effect of human gastric juice on the reticulocytes of albino rats. Am. J. Digest. Dis. 7: 277–280 (1940).

SHABAD, L. M.: Production experimentale de tumeurs malignes par un extrait benzenique due foie d'un cancéreux . . . Compt. rend. Soc. de biol. 124: 213–216 (1937).

STEINER, P. E.: Cancer-producing agents from human sources. Internat. Abstr. Surg. 76: 105–112 (1943).

STRANDELL, B.: Pernicious anemia; a study of 117 cases. Acta med. scandinav., suppl. 40, 124 pp. (1931).

STURGIS, C. C., and GOLDHAMER, S. M.: Macrocytic anemia, other than pernicious anemia, associated with lesions of the gastrointestinal tract. Ann. Int. Med. 12: 1245–1262 (1939).

VELDE, G.: Die Beziehungen zwischen perniziöser Anämie, Magenpolypen und Magencarcinom. Ztschr. f. klin. Med. 134: 653–670 (1938).

WASHBURN, R. N., and ROZENDAAL, H. M.: Gastric lesions associated with pernicious anemia. Ann. Int. Med. 11: 2172–2180 (1938).

WHITE, R. R.: Simultaneous carcinoma and tuberculosis of the stomach in a case of pernicious anemia. Proc. Staff Meet., Mayo Clin. 18: 165–172 (1943).

WILKINSON, J. F.: Pernicious anaemia and malignant disease. Acta med. scandinav. 80: 466–479 (1933).

WILKINSON, J. F.: Pernicious anemia and cancer. Brit. M. J. 2: 662 (1945).

ZADIK, I.: Todesfälle im Remissionsstadium der perniziösen Anämie. Klin. Wchnschr. 8: 1527–1530 (1929).

ACHLORHYDRIA:

ALVAREZ, W. C.: Discoverer of hypochlorhydria in cases of cancer of the stomach. Am. J. Digest. Dis. 7: 540 (1940).

ANDERSEN, A. C., SLUTZKY, B., and MAERTZ, R. W.: Stomach acidity effect of pedicle jejunal graft in wall of stomach. Gastroenterology 4: 323–331 (1945).

ANDRUS, W. DE W., LORD, J. W. JR., and STEFKO, P.: Physiology of secretion; a possible mode of action of pedicle jejunal graft on gastric secretion as indicated by changes in pH of the surface of the mucosa of the stomach. Am. J. Physiol. 141: 75–77 (1944).

ASK-UPMARK, E.: On the gastric acidity in cancer of the stomach. Acta chir. scandinav. 80: 93–102 (1937).

ATKINSON, A. J., and IVY, A. C.: Further attempts to produce achlorhydria. Am. J. Digest. Dis. 5: 30–35 (1938).

BABKIN, B. P., KOMAROV, O., and KOMAROV, S. A.: Effect of activated ergosterol and of parathyroid hormone on gastric secretion in the dog. Endocrinology 26: 703–715 (1940).

BEAZELL, J. M., and IVY, A. C.: The influence of alcohol on the digestive tract; review. Quart. J. Studies on Alcohol 1: 45–73 (1940).

BENNETT, T. I., and RYLE, J. A.: Studies in gastric secretion; study of normal gastric function based on investigation of 100 healthy men by means of fractional method of gastric analysis. Guy's Hosp. Rep. 71: 286 (1921).

BLOOMFIELD, A. L.: The mechanism of decrease of gastric secretion with advancing years. Am. J. M. Sc. 190: 325–330 (1935).

BLOOMFIELD, A. L., and POLLAND, W. S.: Diagnostic value of studies of gastric secretion. J. A. M. A. 92: 1508–1513 (1929).

BLOOMFIELD, A. L., and POLLAND, W. S.: Gastric anacidity, its relation to disease. 188 pp. Macmillan Co., New York (1933).

BLOOMFIELD, A. L., and POLLAND, W. S.: The fate of people with unexplained gastric anacidity; follow-up studies. J. Clin. Investigation 14: 321–324 (1935).

BULL, H. B., and GRAY, J. S.: Secretion of hydrocloric acid by the stomach. Gastroenterology 4: 175–182 (1945).

BROWN, R. B., PENDERGRASS, E. P., and BURDICK, E. D.: Gastrointestinal tract in hyperthyroidism. Surg., Gynec. & Obst. 73: 766–783 (1941).

BRUNSCHWIG, A., and CLARKE, T. H.: Histologic structure of carcinomas of the stomach and quality of gastric secretions. Ann. Int. Med. 13: 1001–1004 (1939).

BRUNSCHWIG, A., CLARKE, T. H., VAN PROHASKA, J., and SCHMITZ, R. L.: Secretory depressant in achlorhydric gastric juice of patients with cancer of the stomach. Surg., Gynec. & Obst. 70: 25–30 (1940).

BRUNSCHWIG, A., CLARKE, T. H., VAN PROHASKA, J., and SCHMITZ, R. L.: A gastric secretory depressant in ex-

tracts of achlorhydric carcinomatous stomach. Ann. Surg. 113: 41–46 (1941).

BRUNSCHWIG, A., RASMUSSEN, R. A., CAMP, E. J., and MOE, R.: Gastric secretory depressant in gastric juice. Surgery 12: 887–891 (1942).

BRUNSCHWIG, A., SCHMITZ, R. L., and RASMUSSEN, R.: Experimental observations on achlorhydria of gastric cancer. J. Nat. Cancer Inst. 1: 481–488 (1941).

BRUNSCHWIG, A., SCHMITZ, R. L., and SLOTTIN, L.: The secretion of chlorine ions in achlorhydric gastric juice; observations by means of radioactive chlorine. Am. J. Digest. Dis. 8: 171–173 (1941).

BRUNSCHWIG, A., VAN PROHASKA, J., CLARKE, T. H., and KANDEL, E. V.: Secretory depressant in the gastric juice of patients with pernicious anemia. J. Clin. Investigation 18: 415–422 (1939).

CAREY, J. B., WETHERBY, M., and YLVISAKER, R. S.: Gastric observations in achlorhydria. Am. J. Digest. Dis. 8: 401–407 (1941).

CASE, J. T., and BOLDYREFF, W. N.: Influence of roentgen rays upon gastric secretion. Am. J. Roentgenol. 19: 61–70 (1928).

CHANG, H. C., and SLOAN, J. H.: Influence of experimental hypothyroidism upon gastric secretion. Am. J. Physiol. 80: 732–734 (1927).

CHITRE, R. G.: Anacidity in gastric cancer. Indian Med. Gaz. 79: 472–475 (1944).

COMFORT, M. W., BUTSCH, W. L., and EUSTERMAN, G. B.: Observations on gastric acidity before and after development of carcinoma of the stomach. Am. J. Digest. Dis. & Nutrition 4: 673–681 (1937).

COMFORT, M. W., and VANZANT, F. R.: Gastric acidity in carcinoma of the stomach. Am. J. Surg. 26: 447–456 (1934).

COWGILL, G. R., and GILMAN, A.: Physiology of vitamins. XXIII. The effect of lack of the vitamin B complex on the secretion of gastric juice in dogs with gastric pouches. Arch. Int. Med. 53: 58–70 (1934).

CULMER, C. U., ATKINSON, A. J., and IVY, A. C.: Hydrogen peroxide as a depressant of gastric acidity. Am. J. Digest. Dis. 4: 219–223 (1937).

CULMER, C. U., ATKINSON, A. J., and IVY, A. C.: Depression of gastric secretion by extracts of pregnancy urine. Am. J. Physiol. 126: 472–473 (1939).

CULMER, C. U., GRAY, J. S., ATKINSON, J. L., and IVY, A. C.: On the origin of urogastrone. Science 91: 147–148 (1940).

DAVENPORT, H. W., and FISHER, R. B.: The mechanism of the secretion of acid by the gastric mucosa. Am. J. Physiol. 131: 165–175 (1940).

DYER, H. M., and ROE, J. H.: The relation of nutrition to gastric function. IV. The effect of vitamin A deficiency. Am. J. Digest. Dis. 8: 333–337 (1941).

FABER, K.: Chronic gastritis; its relation to achylia and ulcer. Lancet 213: 901–907 (1927).

FABER, K.: Gastritis and its consequence. 119 pp. Oxford Univ. Press, London (1935).

FAULEY, G. B., STRAUSS, H. A., and IVY, A. C.: Studies on the effect of subtotal gastric resection in the dog. Am. J. Surg. 17: 427–433 (1932).

FERGUSON, J. H., MACFAYDAN, J., and SMITH, E. R. B.: Pilocarpine and gastric anacidity in monkeys. J. Physiol. 82: 1–10 (1934).

FÖLDES, F., and VAJDA, G.: Effect of vitamin A on the secretion of gastric juice in deficient hydrochloric acid production. Brit. M. J. 1: 317–318 (1941).

FRIEDMAN, M. H. F., and SANDWEISS, D. J.: The gastric secretory depressant in urine. Am. J. Digest. Dis. 8: 366–371 (1941).

FRIEDMAN, M. H. F., SANDWEISS, D. J., RECKNAGEL, R. O., and PATTERSON, J. L.: Effects of extracts of urine from pernicious anemia and gastric cancer patients on gastric secretion. Anat. Rec. 75 (suppl.): 53 (1939).

GOLDING, F. C.: The association of atrophic gastritis with hypothyroidism; a preliminary report of 11 cases. Ann. Int. Med. 17: 828–834 (1942).

GRANT, R.: Calcium in gastric mucus and regulation of gastric acidity. Am. J. Physiol. 135: 496–503 (1942).

GRAY, J. S.: Present status of urogastrone. Am. J. Digest. Dis. 8: 365–366 (1941).

GRAY, J. S., WIECZOROWSKI, E., and IVY, A. C.: Inhibition of gastric secretion by extracts of normal male urine. Science 89: 489–490 (1939).

GROSSMAN, M. I., DUTTON, D. F., and IVY, A. C.: An attempt to confirm the alleged inhibitory effect on gastric secretion of jejunal pedicle graft in the wall of the stomach. Surgery 17: 685–692 (1945).

GROSSMAN, M. I., GREENGARD, H., DUTTON, D. F., and WOOLLEY, J. R.: The effect of prolonged administration of enterogastrone on gastric secretion in normal and Mann-Williamson dogs. Gastroenterology 2: 437–441 (1944).

HARTMAN, H. R.: The prognostic value of gastric acidity in cases of resectable carcinoma. J. A. M. A. 83: 1976–1977 (1924).

HELMER, O. M.: Relation of the secretion of mucus to acidity of gastric juice. Am. J. Physiol. 110: 28–36 (1934).

HERRIN, R. C.: Gastric emptying time and acidity in avitaminosis A in dogs. Am. J. Digest. Dis. 7: 164–166 (1940).

HOLMAN, C. W., and SANDUSKY, W. R.: Further observations on the diagnosis and treatment of gastric lesions. Ann. Surg. 112: 339–343 (1940).

HURST, A. F.: Achlorhydria and achylia, and their connection with the Addison's anaemia-subacute combined degeneration syndrome and simple (non-Addisonian) achlorhydric anemia. Quart. J. Med. 1: 157–177 (1932).

HURST, A. F.: The clinical importance of achlorhydria. Brit. M. J. 2: 665–669 (1934).

IVY, A. C.: Digestive system. *In* Cowdry, E. V., Problems of Ageing; Biological and Medical Aspects. Ed. 2, ch. 10. Williams & Wilkins, Baltimore (1942).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

IVY, A. C., and FARRELL, J. I.: The experimental production of achylia gastrica in the dog. Proc. Soc. Exper. Biol. & Med. 23: 752 (1926).

IVY, A. C., ORNDORF, B. H., JACOBY, A., and WHITLOW, J. E.: Studies on the effect of X-rays on glandular activity. III. The effect of X-rays on gastric secretion. Radiology 1: 39–46 (1923).

KIRSNER, J. B., NUTTER, P. B., and PALMER, W. L.: Studies on anacidity: The hydrogen-ion concentration of the gastric secretion, the gastroscopic appearance of the gastric mucosa, and the presence of a gastric secretory depressant in patients with anacidity. J. Clin. Investigation 19: 619–625 (1940).

KOLOUCH, F., JR., DUBUS, A. T. S., and WANGENSTEEN, O. H.: Symposium on ulcer problem; pedicled jejunal transplant onto gastric wall; evaluation of its effect upon gastric acidity and failure of such transplants to afford protection against ulcer provoked by histamine in beeswax. Surgery 17: 667–685 (1945).

LAYNE, J. A., and BERGH, G. S.: The effect of ligation of arteries of the stomach upon acid gastric secretion and upon the endoscopic appearance of the gastric mucosa in the dog. Surgery 13: 136–141 (1943).

LAYNE, J. A., and CAREY, J. B.: The effect of blacktongue-producing diet upon the endoscopic appearance of the gastric mucosa in the dog. Gastroenterology 2: 133–141 (1944).

LEVIN, A. E., and KUCHUR, B. A.: Investigation of relatives of patients with gastric cancer. Lancet 1: 204–205 (1937).

MCNEER, G.: Cancer of the stomach in the young. Am. J. Roentgenol. 45: 537–550 (1941).

MOORE, B., ALEXANDER, W., KELLY, R. E., and ROAF, H. E.: On the absence or marked diminution of free HCl in the gastric contents, in malignant diseases of organs other than the stomach. Lancet 1: 1120–1124 (1905).

MORLEY, J.: Carcinoma of the stomach. Brit. M. J. 2: 949–953 (1937).

DE MURO, P., and MARCONI, F.: The testicle and gastric secretion. Rev. Gastroenterol. 12: 363–368 (1945).

OLIVER, T. H., and WILKINSON, J. T.: Achlorhydria. Quart. J. Med. 2: 431–462 (1933).

PALMER, W. L., and TEMPLETON, F. E.: Effect of radiation therapy on gastric secretion. Tr. Assoc. Am. Physicians 53: 111–114 (1938).

PALMER, W. L., and TEMPLETON, F. E.: Effect of radiation therapy on gastric secretion, J. A. M. A. 112: 1429–1434 (1939).

PHILLIPS, K.: Studies on the neutralization test of gastric acidity in relation to general disease. Am. J. Digest. Dis. and Nutrition 4: 87–91 (1937).

QUIGLEY, J. P.: Enterogastrone—Significant steps in development of the present conceptions. Am. J. Digest. Dis. 8: 363–364 (1941).

RASMUSSEN, R. A., and BRUNSCHWIG, A.: Peptic activity of achlorhydric human gastric juice from carcinomatous stomachs; comparative study. Proc. Soc. Exper. Biol. & Med. 46: 289–300 (1941).

RHOADS, C. P.: Gastric anacidity as a sequel to gastritis particularly the gastritis of pernicious anemia. J. Nat. Cancer Inst. 1: 511–522 (1941).

ROBERTSON, J. D.: Development of achlorhydria and pyloric stenosis in cases of carcinoma of the stomach. Lancet 1: 1495–1497 (1935).

SALTZSTEIN, H. C., and KURTZ, I. J.: The effect of pedicle jejunal transplants in the stomach of Mann-Williamson dogs. Surg., Gynec. & Obst. 82: 194–198 (1946).

SANGSTER, W., GROSSMAN, M. I., IVY, A. C.: The effect of two new histamine antagonists (benadryl and compound 63) on histamine stimulated gastric secretion in the dog. Gastroenterology 6: 436–438 (1946).

SANDWEISS, D. J.: Enterogastrone, anthelone, and urogastrone; a review of the literature and a suggestion for the organization of a national committee for the study of "peptic" ulcer. Gastroenterology 5: 404–415 (1945).

SCHIFFRIN, M. J., and GRAY, J. S.: The effect of urogastrone on gastric secretion in enterectomized dogs. Am. J. Physiol. 137: 417–420 (1942).

SCHINDLER, R., NUTTER, P. B., GROOM, H. E., and PALMER, W. L.: Anatomic foundation of anacidity; gastroscopic study. Arch. Int. Med. 66: 1060–1078 (1940).

SCHNEDORF, J. G., and IVY, A. C.: Failure of histamine and "Gastrin" to provoke secretion in monkeys with anacidity. Proc. Soc. Exper. Biol. & Med. 36: 192–193 (1937).

SCOTT, V. B., MOE, R., and BRUNSCHWIG, A.: Further studies on properties of gastric secretory depressant in gastric juice. Proc. Soc. Exper. Biol. & Med. 52: 45–46 (1943).

SHAY, H., GERSHON-COHEN, J., and FELS, S. C.: Gastric anacidity; its physiologic and clinical significance and its management. Am. J. Digest. Dis. 8: 115–123 (1941).

SHAY, H., KOMAROV, S. A., GRUENSTEIN, M., and FELS, S. S.: The effect of thiamin deprivation upon gastric secretion in the rat. Gastroenterology 6: 199–212 (1946).

SMITH, C. S., WIKOFF, H. L., and SOUTHARD, M. E.: Gastric acidity in apparently healthy individuals. Am. J. Digest. Dis. 12: 117–119 (1945).

SNELL, A. M., and BOLLMAN, J. L.: Gastric secretion following irradiation of the exposed stomach and upper abdominal viscera by roentgen rays. Am. J. Digest. Dis. & Nutrition 1: 164–168 (1934).

SOMERVELL, T. H.: Physiological gastrectomy . . . Brit. J. Surg. 33: 146–152 (1945).

STALKER, L. K., BOLLMAN, J. L., and MANN, F. C.: Effect of cinchophen or the gastric secretion; an experimental study. Arch. Surg. 34: 1172–1178 (1937).

STREET, H. R., COWGILL, G. R., and ZIMMERMAN, H. M.: Some observations of vitamine B6 deficiency in the dog. J. Nutrition 21: 275–290 (1941).

TUERKISCHER, E., and WERTHEIMER, E.: Adrenalectomy and gastric secretion. J. Endocrinology 4: 143–151 (1945).

VANZANT, F. R., ALVAREZ, W. C., EUSTERMAN, G. B., DUNN, H. L., and BERKSON, J.: The normal range of gastric acidity from youth to old age. Arch. Int. Med. 49: 345–359 (1932).

VELDE, G.: Die Beziehungen zwischen perniziöser Anämie, Magenpolypen und Magencarcinom. Ztschr. f. klin. Med. 134: 653–670 (1938).

WAY, S.: Relation between gastric acidity and the anterior pituitary-like hormone content of urine in pregnant women. Brit. M. J. 2: 182–184 (1945).

WIECZOROWSKI, E., GRAY, J. S., and IVY, A. C.: The effect of urogastrone on the gastric secretion in man. Am. J. Physiol. 129: 496 (1940).

WILHELMJ, C. M., FINEGAN, R. W., and HILL, F. C.: The physiological control of gastric acidity. Am. J. Digest. Dis. 4: 547–550 (1937).

WOLMAN, I. J.: Digestive secretions in infancy and childhood. Am. J. M. Sc. 206: 770–794 (1943).

GASTRIC ULCER:

ABRAHAMSON, R. H., CHURCH, R., and HINTON, J. W.: Hormone effect on the male gastroduodenal mucosa. Am. J. M. Sc. 204: 809–823 (1942).

ALLEN, A. W.: Gastric ulcer and cancer. Surgery 17: 750–754 (1945).

ALLEN, A. W., and WELCH, C. E.: Gastric ulcer; the significance of this diganosis and its relationship to cancer. Ann. Surg. 114: 498–509 (1941).

ALLEN, C. I.: Primary carcinoma of the duodenum. Am. J. Surg. 40: 89–101 (1938).

BACHRACH, W. H., SCHMIDT, C. R., and BEAZELL, J. M.: The relation of bile and pancreatic juice to duodenal ulcer in dogs. Proc. Soc. Exper. Biol. & Med. 40: 322–324 (1939).

BAUER, J.: The relation between peptic ulcer and cancer of the stomach from the genetic point of view. Rev. Gastroenterol. 7: 21–24 (1940).

BEAZELL, J. M., and IVY, A. C.: Chronic gastric ulcer following bilateral vagotomy in the rabbit and in the dog. Arch. Path. 22: 213–219 (1936).

BERG, B. N.: Pathological changes in nutritional gastritis in rats. Am. J. Path. 18: 49–61 (1942).

BLOOMFIELD, A. L., CHEN, K. C., and FRENCH, L. R.: Basal gastric secretion as a clinical test of gastric function with special reference to peptic ulcer. J. Clin. Investigation 19: 863–869 (1940).

BOARD, G. G., and BERMAN, L. G.: Treatment of experimental Mann-Williamson ulcers with anterior pituitary-like hormone (antuitrin-S). Am. J. Digest. Dis. 8: 27–28 (1941).

BROWN, G. E., Jr., and RIVERS, A. B.: A comparison of gastric acidity among men and women suffering from duodenal ulcers. . . Am. J. Digest. Dis. 12: 33–36 (1945).

COMFORT, M. W., and VANZANT, F. R.: Gastric acidity in carcinoma of the stomach. Am. J. Surg. 26: 447–456 (1934).

DODDS, E. C., NOBLE, R. L., SCARFF, R. W., and WILLIAMS, P. C.: Pituitary control of alimentary blood flow and secretion; changes in the stomach produced by administration of posterior pituitary extract. Proc. Roy. Soc. London, s. B, 123: 22–26 (1937).

DRAGSTEDT, L. R.: Pathogenesis of gastroduodenal ulcers. Arch. Surg. 44: 438–451 (1942).

EUSTERMAN, G. B.: Carcinomatous gastric ulcer; misleading results of medical therapy. J. A. M. A. 118: 1–5 (1942).

EWING, J.: Etiologic indications of early gastric cancer. Rev. Gastroenterol. 7: 305–309 (1940).

GRAY, H. K.: Carcinoma of the stomach, with particular reference to the significance of persistent symptoms ascribed to the stomach and the malignant potentiality of gastric ulcers. J. A. M. A. 116: 22–25 (1941).

GROSSMAN, M. I., DUTTON, D. F., and IVY, A. C.: An attempt to prevent histamine induced ulcers in dogs by the administration of enterogastrone concentrates. Gastroenterology 6: 145–146 (1946).

HINTON, J. W.: Carcinoma of the stomach emphasizing some of the misconceptions of the disease. Bull. New York Acad. Med. 17: 820–843 (1941).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

JUDD, E. S., and PRIESTLEY, J. T.: Treatment of gastric ulcer. Surg., Gynec. & Obst. 77: 21–25 (1943).

KIERNAN, P. C., and LARSON, R.: Small ulcerating lesion of the stomach; report of two cases. Proc. Staff Meet., Mayo Clin. 21: 218–224 (1946).

KIRKLIN, B. R.: Mistakes and misunderstandings in the roentgenologic diagnosis of gastric cancer. Arch. Surg. 46: 861–864 (1943).

KOLOUCH, F., JR.: A direct technique for studying chemical and other injury to exposed mucosal surfaces. Surgery 17: 641–644 (1945).

MALLORY, T. B.: Carcinoma in situ of the stomach and its bearing on the histogenesis of malignant ulcers. Arch. Path. 30: 348–362 (1940).

METZ, M. H. and LACKEY, R. W.: The treatment of peptic ulcer with posterior pituitary extracts. Texas State J. Med. 32: 589–590 (1937).

MORLEY, J.: The surgical pathology of peptic ulcer and gastric carcinoma. Edinburgh M. J. 53: 273–283 (1946).

NECHELES, H.: Enterogastrone, urogastrone, and anthelone. (Editorial). Gastroenterology 5: 427–429 (1945).

NEUWELT, F., and NECHELES, H.: Experimental cinchophen ulcer. Proc. Soc. Exper. Biol. & Med. 44: 78–81 (1940).

PALMER, W. L., and HUMPHREYS, E. M.: Gastric carcinoma; observation on peptic ulceration and healing. Gastroenterology 3: 257–272 (1944).

ROTH, J. A., and IVY, A. C.: The experimental production of acute and subacute gastric ulcers in cats by the intramuscular injection of caffeine in beeswax. Gastroenterology 2: 274–285 (1944).

SANDWEISS, D. J.: Entergastrone, anthelone, and urogastrone; a review of the literature and a suggestion for the organization of a national committee for the study of "peptic" ulcer. Gastroenterology 5: 404–415 (1945).

SCHINDLER, R: Gastroscopy; the endoscopic study of gastric pathology. 343 pp. Univ. Chicago Press, Chicago (1937).

SCOTT, W. J. M., and MIDER, G. B. Malignancy in the chronic gastric ulcer. Am. J. Surg. 40: 42–50 (1938).

SHAY, H., KOMAROV, S. A., GRUENSTEIN, M., SIPLET, H., and MERANZE, D.: A method for the uniform production of experimental gastric ulcer in the rat. Am. J. M. Sc. 209: 549 (1945).

STALKER, L. K., BOLLMAN, J. L., MANN, F. C.: Experimental peptic ulcers produced by cinchophen. Proc. Staff Meet., Mayo Clin., 11: 695–698 (1936).

STEWART, M. J.: The histological criteria of ulcer-cancer of the stomach. J. Path. & Bact. 29: 321–324 (1926).

STOUT, A. P.: Pathology of carcinoma of the stomach. Arch. Surg. 46: 807–822 (1943).

WILSON, L. B., and MACCARTY, W. C.: The pathological relationships of gastric ulcer and gastric carcinoma. Tr. Assoc. Am. Physicians 24: 593–602 (1909).

WOLF, S., and WOLFF, H. G.: Human gastric function; an experimental study of a man and his stomach. 195 pp. Oxford Univ. Press, New York, London, and Toronto (1943).

POLYPS:

ANDERVONT, H. B.: Development and genetic characteristics of the adenomatous stomach lesion in strain I mice. Pub. Health Rep. 54: 1851–1855 (1939).

ANDERVONT, H. B., and STEWART, H. L.: Adenomatous lesion in the stomach of strain I mice. Science 86: 566 (1937).

BENEDICT, E. B., and ALIEN, A. W.: Adenomatous polypi of the stomach, with special reference to malignant degeneration. Surg., Gynec. & Obst. 58: 79–84 (1934).

BRUNN, H., and PEARL F.: Multiple gastric polyposis. Am. J. Surg. 40: 51–54 (1938).

GOODPASTURE, E. W.: An anatomical study of senescence in dogs, with especial reference to the relation of cellular changes of age to tumors. J. M. Research 38: 127–189 (1918).

MILLER, T. G., ELIASON, E. L., and WRIGHT, V. M.: Carcinomatous degeneration of polyp of the stomach; report of 8 personal cases with review of 24 recorded by others. Arch. Int. Med. 46: 841–878 (1930).

PEARL, F. L., and BRUNN, H.: Multiple gastric polyposis; supplementary report of 41 cases, including 3 new personal cases. Surg., Gynec. & Obst. 76: 257–281 (1943).

STEWART, M. J.: Observations on the relation of malignant disease to benign tumors of the intestional tract. Brit. M. J. 2: 567–569 (1929).

WESTHUES, H.: Entstehung des Magencarcinoms aus Gastritis und entzündlichen Polypen? Arch. f. klin. Chir. 203: 391–435 (1942).

DIAGNOSIS:

ABRAHAMSON, R. H., and HINTON, J. W.: Carcinoma of the stomach; review of 444 cases to emphasize inadequacy of present methods for early diagnosis. Surg., Gynec. & Obst. 71: 135–141 (1940).

ALLEN, A. W., and WELCH, G. E.: Gastric ulcer; the significance of this diagnosis and its relationship to cancer. Ann. Surg. 114: 498–509 (1941).

ALVAREZ, W. C.,: How early do physicians diagnose cancer of the stomach in themselves? Study of history of 41 cases. J. A. M. A. 97: 77–83 (1931).

BADE, H.: Hat die Röntgendiagnostik eine Vergesserung der Heilungsergebnisse des Magenkrebses gebracht, und besteht überhaupt diese Möglichkeit? Forstschr. a. d. Geb. d. Röntgenstrahlen 62: 114–122 (1940).

BENAVIDES, A., and GUTIERREZ, O.: El gastrotonometro del Dr. Gaultier en la exploración fisiopatológica del estomago. Prensa méd., La Paz 2: 235–238 (1942).

BLOOMFIELD, A. L., CHEN, K. C., and FRENCH, L. R.: Basal gastric secretion as a clinical test of gastric function with special reference to peptic ulcer. J. Clin. Investigation 19: 863–869 (1940).

BLOOMFIELD, A. L., and POLLAND, W. S.: Diagnostic value of studies of gastric secretion. J. A. M. A. 92: 1508–1513 (1929).

BOLEN, H. L.: Diagnostic value of blood studies in malignancy of the gastro-intestinal tract. Am. J. Surg. 63: 316–323 (1944).

BOZLER, E.: The action potentials of the stomach. Am J. Physiol. 144: 693–700 (1945).

CHAMOREO- R., SALINAS, R.: Sobre una prueba funcional gastrica. Rev. clín. españ. 3: 139–140 (1941).

COMFORT, M. W., and VANZANT, F. R.: Gastric acidity in carcinoma of the stomach. Am. J. Surg. 26: 447–456 (1934).

CYTRONBERG, S.: Early diagnosis of gastric cancer. Rev. Gastroenterol. 7: 391–399 (1940).

DAILEY, M. E., and MILLER, E. R.: A search for symptomless gastric cancer in 500 apparently healthy men of forty-five and over. Gastroenterology 5: 1–4 (1945).

EUSTERMAN, G. B.: Carcinomatous gastric ulcer; misleading results of medical therapy. J. A. M. A. 118: 1–5 (1942)

FEROS, J. M.: Study on the electro-cardiogastrographic test. Rev. Gastroenterol. 12: 99–110 (1945).

FRISHMAN, R. L., and GORIN, M. G.: The diagnostic significance of examination of stomach washing in patients suspected of having cancer of stomach. Klin. Med. 20: 59–67 (1942).

GARREY, W. E.: An evaluation of peritoneoscopy, with particular reference to the diagnosis of abdominal tumors. New England J. Med. 225: 180–184 (1941).

GILL, A. M.: Evaluation of gastroscopy; analysis of 1,000 examinations. Lancet 1: 333–334 (1943).

GOLOB, M.: Gastric carcinoma; review of errors in diagnosis. Am. J. Digest. Dis. 13: 17–23 (1946).

GRACIA PAREDES, A., and FONT PUIGDERRAJOLS, J.: Diagnóstico del cancer gastrico, valor de la prueba del azul de metileno. Medicina españ. 10: 412–418 (1943).

GRAY, H. K.: Carcinoma of the stomach, with particular reference to the significance of persistent symptoms ascribed to the stomach and the malignant potentiality of gastric ulcers. J. A. M. A. 116: 22–25 (1941).

HOLLANDER, F.: The measurement of electrical potentials in the stomach. Gastroenterology 3: 319–322 (1944).

HOLMAN, C. W., and SANDUSKY, W. R.: Further observations on the diagnosis and treatment of gastric lesions. Ann. Surg. 112: 339–343 (1940).

HOON, M. R.: Carcinoma of the stomach, a plea for early diagnosis and surgical treatment. Pennsylvania M. J. 46: 929–932 (1942–43).

IACOBOVICI, I., BALTACEANU, G., COMANESCO, A., and VASILESCO, G. E. M.: Researches on the vitamin C content of the gastric wall in different affections of the stomach. Bull. Acad. de méd. de Roumanie 17: 187–194 (1945).

JEKEL, L. G.: Diagnosis of carcinoma of the stomach from a fragment of the tumor obtained during routine gastric analysis. J. Lab. & Clin. Med. 21: 836–838 (1936).

JUDD, E. S., and PRIESTLEY, J. T.: Treatment of gastric ulcer. Surg., Gynec. & Obst. 77: 21–25 (1943).

KIERNAN, P. C., and LARSON, R.: Small ulcerating lesion of the stomach; report of two cases. Proc. Staff Meet., Mayo Clin. 21: 218–224 (1946).

KIRKLIN, B. R.: Mistakes and misunderstandings in the roentgenologic diagnosis of gastric cancer. Arch. Surg. 46: 861–864 (1943).

KOZAWA, S., FUKUSHIMA, K., OKADA, J., and FURUKAWA, G.: Electrogastrogram; a method for the early diagnosis of stomach cancer. Collect. Papers, Fac. Med., pp. 47–52 (1938). Osaka (1939).

MACCARTY, W. C.: Early cancer of the gastro-intestinal tract. Am. J. Path. (Scient. Proc., Assoc. Path. & Bact.) 17: 630 (1941).

MOSTO, D., and POLAK, M.: Valor del examen histopatólogico del jugo gástrico incluido en parafina, en el diagnóstico del cancer de estómago. Día méd. 10: 295 (1938).

MULLEN, T. F.: Some factors influencing the curability of cancer of the stomach. Surg., Gynec. & Obst. 72: 298–306 (1941).

MULROONEY, R. E.: Cytology of gastric contents in reference to gastritis. Proc. Staff Meet., Mayo Clin. 15: 81–85 (1940).

MULROONEY, R. E., and EUSTERMAN, G. B.: Cytology of the gastric contents, with special reference to gastritis. Arch. Surg. 42: 55–70 (1941).

PALMER, W. L., and HUMPHREYS, E. M.: Gastric carcinoma; observations on peptic ulceration and healing. Gastroenterology 3: 257–272 (1944.)

PAPANICOLAOU, G. N.: Diagnostic value of exfoliated cells from cancerous tissues. J. A. M. A. 131: 372–378 (1946).

PHILLIPS, K.: Studies on the neutralization test of gastric acidity in relation to general disease. Am. J. Digest. Dis. & Nutrition 4: 87–91 (1937).

REHM, W. S.: Effect of electric current on gastric secretion and potential. Am. J. Physiol. 144: 115–125 (1945).

REHM, W. S., and ENELOW, A. J.: The effect of thiocyanate on gastric potential and secretion. Am. J. Physiol. 144: 701–705 (1945).

RENSHAW, R. J. F., CLARK, G. E., JR., and FORSYTHE, J. R.: A critical analysis of 938 gastroscopic examinations. Am. J. Digest. Dis. 9: 401–404 (1942).

RIGLER, L. R., KAPLAN, H. S., and FINK D. L.: Pernicious anemia and the early diagnosis of tumors of the stomach. J. A. M. A. 128: 426–432 (1945).

ST. JOHN, F. B., SWENSON, P. C., and HARVEY, H. D.: An experiment in the early diagnosis of gastric carcinoma. Ann. Surg. 119: 225–231 (1944).

SAINZ, P. A., and RODRIQUEZ, A.: El dietetoquimograma. Rev. med. cubana 53: 1033–1040 (1942).

SCHINDLER, R.: Gastroscopy: the endoscopic study of gastric pathology. 343 pp. Univ. Chicago Press, Chicago (1937).

SCOTT, W. J. M., and MIDER, G. B.: Malignancy in the chronic gastric ulcer. Am. J. Surg. 40: 42–50 (1938).

VIACAVA, E. P., and PACK, G. T.: Significance of supraclavicular signal node in patients with abdominal and thoracic cancer; study of 122 cases. Arch. Surg. 48: 109–119 (1944).

WALTERS, W., GRAY, H. K., and PRIESTLEY, J. T.: Carcinoma and other malignant lesions of the stomach. 567 pp. W. B. Saunders Co., Philadelphia (1942).

EXPERIMENTAL CARCINOGENESIS:

ANDERVONT, H. B.: Possible causative factors of a stomach lesion in mice. Pub. Health Rep. 54: 2085–2093 (1939).

BEAZELL, J. M., and IVY, A. C.: Chronic gastric ulcer following bilateral vagotomy in the rabbit and in the dog. Arch. Path. 22: 213–219 (1936).

BECK, S., and PEACOCK, P. R.: Gastro-papillomatosis due to vitamin A deficiency induced by heated fats. Brit. M. J. 2: 81–83 (1941).

BERG, B. N.: Pathological changes in nutritional gastritis in rats. Am. J. Path. 18: 49–61 (1942).

BONNÉ, C., and SANDGROUND, J. H.: On the production of gastric tumors bordering on malignancy, in Javanese monkeys through the agency of Nochtia nochtia, a parasitic nematode. Am. J. Cancer 37: 173–185 (1939).

BRUNSCHWIG, A., and RASMUSSEN, R. A.: Relation of diet to benign neoplasia (ulcero-papillomas) of the rat's stomach. Proc. Inst. Med., Chicago 13: 387–388 (1941).

BULLOCK, F. D., and ROHENBURG, G. L.: Experimental "carcinomata" of animals and their relation to true malignant tumors. J. Cancer Research 3: 227–273 (1918).

DICKENS, F., and WEIL-MALHERBE, H.: Investigation into the possible carcinogenic activity of wood smoke. Cancer Research 2: 680–684 (1942).

FIBIGER, J.: Untersuchungen über eine Nematode (Spiroptera spn. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwulstbildunger in Magen der Ratte hervorzurufen. Ztschr. f. Krebsforsch. 13: 217–280 (1913).

HARDÉ, E.: Cancer gastrique observé chez une souris en expérience. Compt. rend. Soc. de biol. 110: 245–246 (1932).

HOLLANDER, F.: Experimental gastric carcinoma and mucus secretion (Editorial). Gastroenterology 2: 286–288 (1944).

IVY, A. V.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

KIRBY, A. H. M.: Attempts to induce stomach tumors. I. The effect of cholesterol heated to 300° C. Cancer Research 3: 519–525 (1943).

KIRBY, A. H. M.: Attempts to induce stomach tumors. III. The effects of (a) a residue of cholesterol heated to 300° C., and (b) 3, 5-cholestadiene. Cancer Research 4: 94–97 (1944).

KIRBY, A. H. M.: Attempts to induce stomach tumors. IV. The effect of (a) cholesteryl esters heated to 300° C., and (b) cholesterol heated to 430° C. Cancer Research 5: 129–134 (1945).

KLEIN, A. J., and PALMER, W. L.: Experimental gastric carcinoma; critical review with comments on criteria of induced malignancy. Arch. Path. 29: 814–844 (1940).

KOLOUCH, F., JR.: A direct visual technique for studying chemical and other injury to exposed mucosal surfaces. Surgery 17: 641–644 (1945).

LARSEN, C. D., and BARRETT, M. K.: Administration of 3,5-cholestadiene and dicholesteryl ether to mice and rats. J. Nat. Cancer Inst. 4: 587–594 (1944).

LORENZ, E., and STEWART, H. L.: Intestinal carcinoma and other lesions in mice following oral administration of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene. J. Nat. Cancer. Inst. 1: 17–40 (1940).

MAGRUDER, S. R.: Unusual reactions of mucosa following hypophysectomy. Anat. Rec. 73: 269–271 (1939).

PEACOCK, P. R., and KIRBY, A. H. M.: Attempts to induce stomach tumors. II. The action of carcinogenic hydrocarbons on stock mice. Cancer Research 4: 88–93 (1944).

PERRY, I. H., and GINZTON, L. L.: The development of tumors in female mice treated with 1,2,5,6-dibenzanthracene and theelin. Am. J. Cancer 29: 680–704 (1937).

ROFFO, A. H.: Tumeurs malignes développées dans l'appareil digestif par l'ingestion de graisses oxydées par chauffage. Bull. Assoc. franç. p. l'étude du cancer 28: 556–588 (1939).

ROFFO, A. H.: Cancerización gástrica por ingestion de alquitrán tabáquico. Prensa méd argent. 29: 18–35 (1942).

RUSCH, H. P., BAUMANN, C. A., and MAISON, G. L.: Production of internal tumors with chemical carcinogens. Arch. Path. 29: 8–19 (1940).

SCHABAD, L. M.: Action of coal-tar administered per os and per rectum in white mice. (Abstract). Cancer Rev. 3: 116–117 (1928).

SHARPLESS, G. R.: Neoplastic hyperplasia of mucosa in rats. Proc. Soc. Exper. Biol. & Med. 34: 684–685 (1936).

SMITH, G. M.: Morphological changes in tissues with changes in environment. III. Gastric mucous membrane inplanted into the intestine. J. M. Research 33: 337–343 (1916).

SMITH, G. M.: Comment on experimental gastric cancer. Conn. M. J. 8: 409–414 (1944).

STEWART, H. L.: Induction of gastric tumors in strain A mice by methylcholanthrene. Arch. Path. 29: 153–162 (1940).

STEWART, H. L.: Hyperplastic and neoplastic lesions of the stomach in mice. J. Nat. Cancer Inst. 1: 489–509 (1941).

STEWART, H. L., and LORENZ, E.: Adenocarcinoma of the pyloric stomach and other gastric neoplasms in mice inducted with carcinogenic hydrocarbons. J. Nat. Cancer Inst. 3: 175–189 (1942).

STRONG, L. C.: Genetic analysis of induction of tumors by methylcholanthrene. IX. Induced and spontaneous adenocarcinomas of the stomach in mice. J. Nat. Cancer Inst. 5: 339–362 (1945).

STRONG, L. C., COLLINS, V. J., and DURAND, E. A.: Genetic analysis of induction of tumors by methylcholanthrene. IV. Probably remote induction of various types of gastric lesions. Cancer Research 3: 21–28 (1943).

SUGIURA, K.: The relation of diet to the development of gastric lesions in the rat. Cancer Research 2: 770–775 (1942).

VANPROHASKA, J., BRUNSCHWIG, A., and WILSON, H.: Oral administration of methylcholanthrene to mice. Arch. Surg. 38: 328–333 (1939).

VOEGLIN, C.: A program for the study of cancer of the stomach. J. Nat. Cancer Inst. 1: 539–543 (1941).

WOLF, S., and WOLFF, H. G.: Human gastric function: An experimental study of a man and his stomach. 195 pp. Oxford Univ. Press, New York, London, and Toronto (1943).

MISCELLANY:

BABKIN, B. P.: Secretory mechanism of the digestive glands. 900 pp. Paul B. Hoeber, Inc., New York (1944).

BARRETT, M. K., and LARSEN, C. D.: Test of desoxycholic acid for carcinogenicity in rats and mice. J. Nat. Cancer Inst. 4: 595–600 (1944).

BRUNN, H., and PEARL, F.: Multiple gastric polyposis. Am. J. Surg. 40: 51–54 (1938).

COOK, J. W., KENNAWAY, E. L., and KENNAWAY, N. M.: Production of tumours in mice by desoxycholic acid. Nature, London 145: 627 (1940).

CURTH, H. O.: Benign type of acanthosis nigricans: Etiology. Arch. Dermat. & Syph. 34: 353 (1936).

FERREIRA, R.: Da integridade das mucosa gástric e duodenal; da imunidade da parede do estómago em relacão ao poder proteolítico de seu próprio suco. Da úlcera péptica atrofânica. Brasil-méd. 56: 451–456 (1942).

FIESER, L. F., and NEWMAN, M. S.: Methylcholanthrene from cholic acid. J. Am. Chem. Soc. 57: 961 (1935).

GHIRON, V.: Studies in the carcinogenic potency of desoxycholic acid in rats. Proc. 3d Internat. Cancer Cong., p. 116 (1939).

HELMER, O. M.: Relation of the secretion of mucus to acidity of gastric juice. Am. J. Physiol. 110: 28–36 (1934).

HESS, M., and HOLLANDER, F.: Permanent metachromatic staining of gastric mucus smears. J. Lab. & Clin. Med. 29: 321–333 (1944).

HOLLANDER, F.: Experimental gastric carcinoma and mucus secretion. (An editorial). Gastroenterology 2: 286–288 (1944).

HOLLANDER, F.: Physiology of mucus secretion. J. Nat. Cancer Inst. 5: 367 (1945).

HOLLANDER, F., STEIN, J., and LAUBER, F. V.: The consistency, opacity, and columnar cell content of gastric mucus secreted under the influence of several mild irritants. Gastroenterology 6: 576–595 (1946).

HOLLANDER, L.: Acanthosis nigricans, is it a form of avitaminosis? Arch. Dermat. & Syph. 48: 650-655 (1943).

IVY, A. C.: Gastric physiology in relation to gastric cancer. J. Nat. Cancer Inst. 5: 313–330 (1945).

JUDD, E. S., Jr.: Residual lesions of ulcerative gastritis; possible relationship to carcinoma of the stomach. Surg., Gynec. & Obst. 75: 424–432 (1942).

JUDD, E. S., Jr.: Possible relationship of residual lesions of ulcerative gastritis to the development of carcinomas of the stomach. Proc. Staff Meet., Mayo Clin. 14: 52–56 (1939).

ORNDORFF, J. R., FAULEY, G. B., and IVY, A. C.: Prophylactic value of gastric mucin in therapy of postoperative jejunal ulcer. Am. J. Digest. Dis. & Nutrition 3: 26–34 (1936).

POPOFF, N. W.: Epithelial functional rejuvenation observed in the mucous cells of the gastrointestinal tract and the parietal cells of the stomach. Arch. Path. 27: 841–887 (1939).

SCHOCH, D., and FOGELSON, S. J.: Studies on peptic inhibition. Proc. Soc. Exper. Biol. & Med. 50: 304–308 (1942).

STEINER, P. E.: Cancer-producing agents from human sources. Internat. Abstr. Surg. 76: 105–112 (1943).

WIELAND, H., and DANE, E.: Untersuchungen über die Konstitution der Gallensäuren. LII Mitteilung. Über die Haftstelle der Seitenkette. Ztschr. f. physiol. Chem. 219: 240–244 (1933).

WIGODSKY, H. S., BUSSABARGER, R. A., and FOGELSON, S. J.: The effect of gastric mucin on the hemoglobin regeneration in anemic dogs. J. Lab. & Clin. Med. 25: 13–18 (1939).

WILLIAMS, W. L.: The effect of gastric mucin on the growth of transplanted tumor (lymphosarcoma) cells in mice. Yale J. Biol. & Med. 17: 311–318 (1944).

WOLF, S., and WOLFF, H. G.: Human gastric function; an experimental study of a man and his stomach. 195 pp. Oxford Univ. Press, New York, London, and Toronto (1943).

EFFECT OF PROLONGED INGESTION OF THIOUREA ON MAMMARY GLANDS AND THE APPEARANCE OF MAMMARY TUMORS IN ADULT C3H MICE[1,2]

By HAROLD P. MORRIS, *senior nutrition chemist*, CELIA S. DUBNIK, *junior chemist*, and ALBERT J. DALTON, *senior cytologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

It is now recognized that estrogenic hormones play an important part in the hormonal influences affecting the development of spontaneous mammary tumors in mice. It seems probable that the secretions from other endocrine glands, either directly or indirectly, may also affect the incidence of these mammary tumors. Factors which affect mammary-tumor formation may do so by inhibiting mammary-gland development. Gardner (*1*) observed that desiccated thyroid fed to normal male mice caused proliferation of the mammary-gland ducts and hyperplastic end buds, whereas castrated male mice similarly treated showed no mammary growth. The adrenals of both intact and gonadectomized males fed desiccated thyroid were greatly increased in size. Mixner and Turner (*2*) observed that thyroxin increased the efficiency of injected progesterone and estrone in producing lobule-alveolar growth in castrated female mice.

Among the natural plant materials known to produce a goitrogenic effect, Kennedy and Purves (*3*) reported that rats ingesting a diet containing 45 percent *Brassica* seed for 3–4 months not only developed thyroid hyperplasia but also a hypertrophy of the adrenal cortex and a delay in the development of the ovaries. Griesbach (*4*) described histologic changes in the pituitary of rats ingesting *Brassica* seed, which were characterized by an increase in basophils, with hyalinization, vacuolation, formation of "signet ring" cells, and simultaneous loss of acidophils.

Dalton, Morris, and Dubnik (*5*) described histologic changes in the adrenals of mice after prolonged ingestion of thiourea. These changes were characterized by a decrease in the width of the cortex and loss of lipid. Baumann and Marine (*6*) described a similar decrease in width of the adrenal cortex in the rat after prolonged ingestion of thiouracil. The hypertrophy of the adrenal cortex noted by Kennedy and Purves may represent a mild stimulation whereas the decrease in width of the cortex may be due to exhaustion occasioned by longer stimulation.

Pure chemical compounds can now be used to interfere specifically with the normal secretion of the thyroid gland, especially thyroxin formation. These not only simulate the effects produced by the surgical removal of the gland but also avoid the attendant technical difficulties. The term "chemical thyroidectomy" may be used to describe this process. The condition so produced can be maintained only by continuous administration of a goitrogenic compound.

Up to the present time, the investigations concerning the role of hormonal factors in spontaneous mammary carcinogenesis in mice have relied largely on two general methods: (1) The surgical removal of specific endocrine glands; and (2) the administration of gland extracts, or when available, pure hormones. A third or new approach to a study of hormonal influences in mammary tumor genesis could be the use of chemical thyroidectomy.

Since the absence of one endocrine gland will affect the function of other endocrine glands, it seemed probable that prolonged chemical thyroidectomy would disturb the normal balance of the animal. If the mammary gland, which depends upon hormonal stimulation for the attainment of full structural development, is altered in structure by chemical thyroidectomy, then that process would be expected to influence mammary-tumor genesis in mice of a susceptible strain. In order to put this idea to immediate test, old female animals of a susceptible strain but without palpable mammary tumors were selected for this experiment. In such a group of mice, tumors of microscopic size almost certainly would be present in some of the animals. It was thought that if any effects of feeding a goitrogenic compound could be observed in these old animals, the procedure would merit further study.

MATERIALS AND METHODS

Adult virgin C3H female mice, 11 months of age, without palpable tumors were used. Fifty-three animals were divided at random into 2 groups. The control group of 26 mice was placed on a stock diet composed of the following constituents:

Stock diet composition:	*Grams*
Skim milk	22.75
Ground whole hard wheat	61.52
Brewers' dehydrated yeast	4.00
Sodium chloride	1.40
Iron citrate powder	.13
Cod-liver oil	2.00
Corn oil	8.20
Total	100.00

Twenty-seven mice in the experimental or treated group were placed on the same diet to which had been added 5 gm. of thiourea per kilogram. This amount of thiourea was too high since 6 animals died during the first week, and all lost weight. The animals were returned to the stock diet for 3 days until they had regained the weight lost. They were then fed the stock diet containing 2.5 gm. of thiourea per kilogram. No weight loss

[1] Presented in part before the Society of Experimental Biology and Medicine, Washington, D. C., February 7, 1946.
[2] Received for publication August 20, 1946.

was noted on this lower level of feeding. After 13 weeks, the level was increased to 3.75 gm. per kilogram. The animals readily tolerated this concentration of thiourea. In the control group, 1 animal died during the first week, and 2 others were ill and were excluded from further consideration. A total of 23 control and 21 treated animals was finally used.

The animals were housed in individual wire-cloth-bottom cages. Both groups partook of food and water ad libitum. Food consumption and body weight measurements were made at weekly intervals. All mice were palpated twice weekly for the presence of tumors. They were killed when tumors attained a diameter of 1 to 1.5 cm., or when death appeared imminent.

Estrus cycles were studied by taking vaginal smears. A small medicine dropper containing a drop of saline was used to wash out vaginal contents. Smears were started the day that the animals were placed on the experiment and were continued until death. Smears were routinely made early in the morning daily except Sunday.

Whole mounts of mammary glands were prepared, using a modification of the technique reported by Turner and Gomez (*7*). The skin was slit by a mid-ventral incision, folded back, and four areas of skin, each containing a major portion of the anterior or posterior groups of glands, were removed. The piece of skin containing the gland was spread on paper toweling, dermis side against the paper, and fixed in Bouin's solution for 60–90 minutes, depending on the amount of fat in the specimen. The tissue was separated from the paper and washed 12–24 hours in running tap water. It was then dehydrated in the usual manner and placed in xylol until all or most of the fat was removed. After rehydration, the glands were dissected away from the attached skin and stained in hematoxylin for one-half hour to several hours, Conklin's modification (*8*) being used. The muscle fibers and connective tissue were removed from the glands under the dissecting microscope. The glands were differentiated in acid water and blued in a saturated aqueous solution of lithium carbonate, dehydrated, and passed into xylol to remove the last traces of fat. Since some of these preparations were made, Huseby and Bittner (*9*) have published a technique using methyl salicylate as the clearing agent. This modification has been incorporated into the technique used in this laboratory. Glands are cleared in methyl salicylate and mounted in clarite.

RESULTS

Estrus Cycles

Ovarian activity as reflected by the vaginal smear was studied in all animals. The variation in the number of cornified cells was noted, and an animal was considered to be in estrus when the smear showed 100 percent cornified cells or when the cornified cell predominated, but a scattering of leukocyte cells was present.

The incidence and periodicity of estrus cycles is described under four headings: regular (estrus occurred at the usual periodic intervals throughout the period of observation); irregular (estrus occurred throughout the period of observation but at irregular intervals); infrequent (a long initial period of anestrus, followed by a sporadic series of estrus cycles with a return to the anestrus condition); and none (no appearance of estrus during the entire observation period).

Table 1 lists the number of weeks that each animal was observed and the estrus incidence under the four headings described. The data in table 1 are summarized in table 2. The 23 untreated animals fall in the first 2 groups, regular and irregular.

TABLE 1.—*Incidence and periodicity of estrus in individual mice for the period each was studied*

Number of weeks on diet	Control mice	Thiourea-treated mice
2	Irregular [1] Regular do do	Regular.[1]
3	do	
4	do do do	None.[1] Do. Do.
7	Irregular Regular	Regular. None. Do.
10	Regular Irregular	Do.
12	Regular	Irregular
16	do do	Do.
17	Irregular Regular Irregular	
19		Infrequent.[1]
21	Regular	
24	do	Infrequent. Do. Regular. Infrequent.
28	Regular [2]	
29	do.[2]	
30		Infrequent.
32	Irregular	
41		Irregular. None.
44		Infrequent. Irregular.
63		Infrequent.

[1] See text for characterization of these groupings.
[2] Animal in anestrus terminal 6 weeks of life.

TABLE 2.—*Distribution of mice on basis of estrus incidence and periodicity*

Diet	Estrus incidence				Number of mice
	Regular	Irregular	Infrequent	None	
Control	17	6	0	0	23
Control plus thiourea	3	4	7	7	21

while the 21 treated animals are distributed among all 4 groups.

REGULAR ESTRUS

Seventeen control and three thiourea-treated animals were in estrus at periodic intervals, as shown in tables 1 and 2. Two of the seventeen control animals listed in table 1 maintained the anestrous phase for the terminal 6 weeks of life. Both animals during this period, however, were carrying rapidly growing tumors, yet they were losing weight. Table 1 shows that control and treated animals having regular estrus cycles were under observation from 2 to 24 weeks and were 50 to 74 weeks of age.

IRREGULAR ESTRUS

Six control and four treated animals were in estrus throughout the period of observation, but estrus did not occur at regular intervals. In individual animals of this group, the period between one estrus and the next varied in length. Sometimes the interval lasted for approximately 1 week and at other times extended over several weeks.

INFREQUENT ESTRUS

Seven animals ingesting thiourea maintained the anestrus condition for the first 8 weeks of the experiment, as shown in tables 1 and 2. From the ninth through the sixteenth week of treatment, the animals passed through a number of estrus cycles. The number of cycles during this period varied with the individual animal and ranged from four to seven. In the thirteenth week, the level of thiourea in the diet was raised. By the sixteenth week of the experimental period, the animals in this group had again returned to the anestrus state.

NO ESTRUS

Seven mice ingesting thiourea maintained the anestrus state throughout the experimental period. This is shown in tables 1 and 2.

MAMMARY-GLAND STRUCTURE

Whole mounts of mammary glands from 21 animals in each group were examined. Two control animals died during the course of the experiment and because of autolysis were unsuitable for further study. The description of the mammary glands of animals on the control diet for 2–17 weeks is based on a study of 15 animals. Four control animals remained for comparison with animals ingesting thiourea for more than 18 weeks. The descriptions of changes in the mammary glands of treated animals after 2, 7, 17, 24, and 44 weeks of thiourea ingestion are each based on studies of 3–4 animals. The second or third pair of thoracic glands was usually selected for illustration.

The glands of all control animals studied showed the usual individual variation one expects in a group of normal virgin female C3H mice over 1 year of age, but in no case did the control mammary glands show evidence of atrophy that approached the earliest changes observed in the treated animals. The control mammary glands did not show any distinctive variation from animal to animal during the 32 weeks in which they were on the experiment. Therefore, only a brief description of the normal gland at the start of the experiment is given. However, illustrations of glands of control mice killed during the course of the experiment are presented for visual comparison.

The involutionary changes observed in the mammary glands of mice ingesting thiourea for 2 to 63 weeks were progressive and became more pronounced as the treatment continued.

No difference was observed between the mammary glands of the control animals and those of animals ingesting thiourea for 2 weeks; the glands from both groups showed the typical structure of a 1-year-old virgin female C3H mouse. The glandular tree was a complex structure possessing numerous side branches and ducts of smaller caliber with many lateral and terminal end buds, which were round, prominent, and deep staining (fig. 1, *A* and *B*).

The mammary glands of mice ingesting thiourea for 7 weeks showed slight changes. The ducts still showed complex branching, but the lateral and terminal end buds, while present and still numerous, were not so large or prominent. A small number of side branches no longer possessed end buds, and some of the smallest branches had become only blunt projections (fig. 2, *A* and *B*).

The glands of the animals after 17 weeks of treatment showed further and more extensive atrophy, especially in the peripheral regions of each gland (fig. 3, *A* and *B*).

The mammary-gland tree of treated animals killed during the twenty-fourth week of the experiment was very much simplified. End buds were few and inconspicuous. Short, blunt projections, the site of the former small-caliber lateral branches, were very numerous (fig. 4, *A* and *B*).

After 44 weeks of treatment, the involution of the glands had become progressively more pronounced. The total area occupied by the glands was smaller. There were fewer ducts per unit area. The larger main ducts were still present; but the number of smaller side branches was considerably decreased, and their contour was smooth and unbroken by alveolar buds. Ducts of smaller caliber were simple, short, blunt projections of the larger ducts. No terminal buds were present (fig. 5).

The last animal killed after 63 weeks of treatment showed essentially the same simplified structure but even more pronounced (fig. 6) than the condition described after 44 weeks of treatment. No normal animal was available for comparison

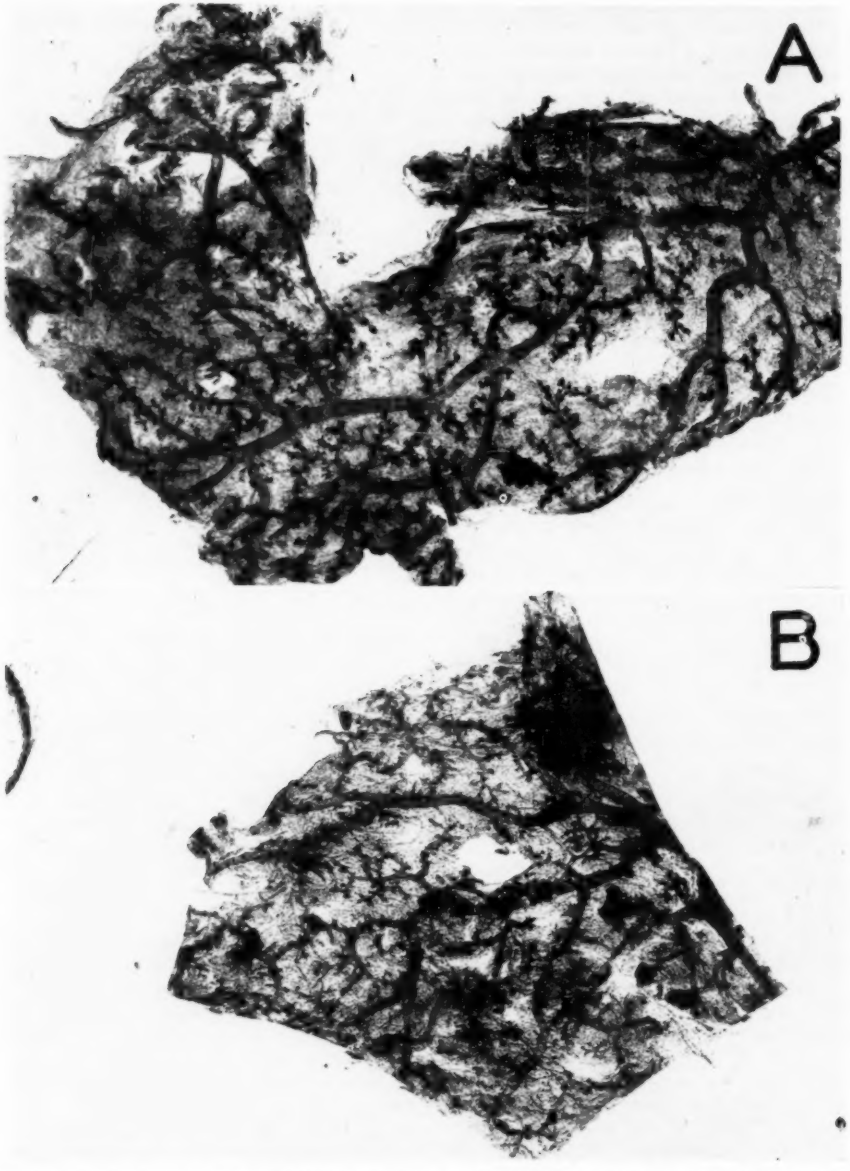

FIGURE 1.—Portions of mammary glands of C3H mice 50 weeks of age. Both show typical adult structure. A, Control animal. × 10. B, Treated animal after 2 weeks of thiourea ingestion. × 10.

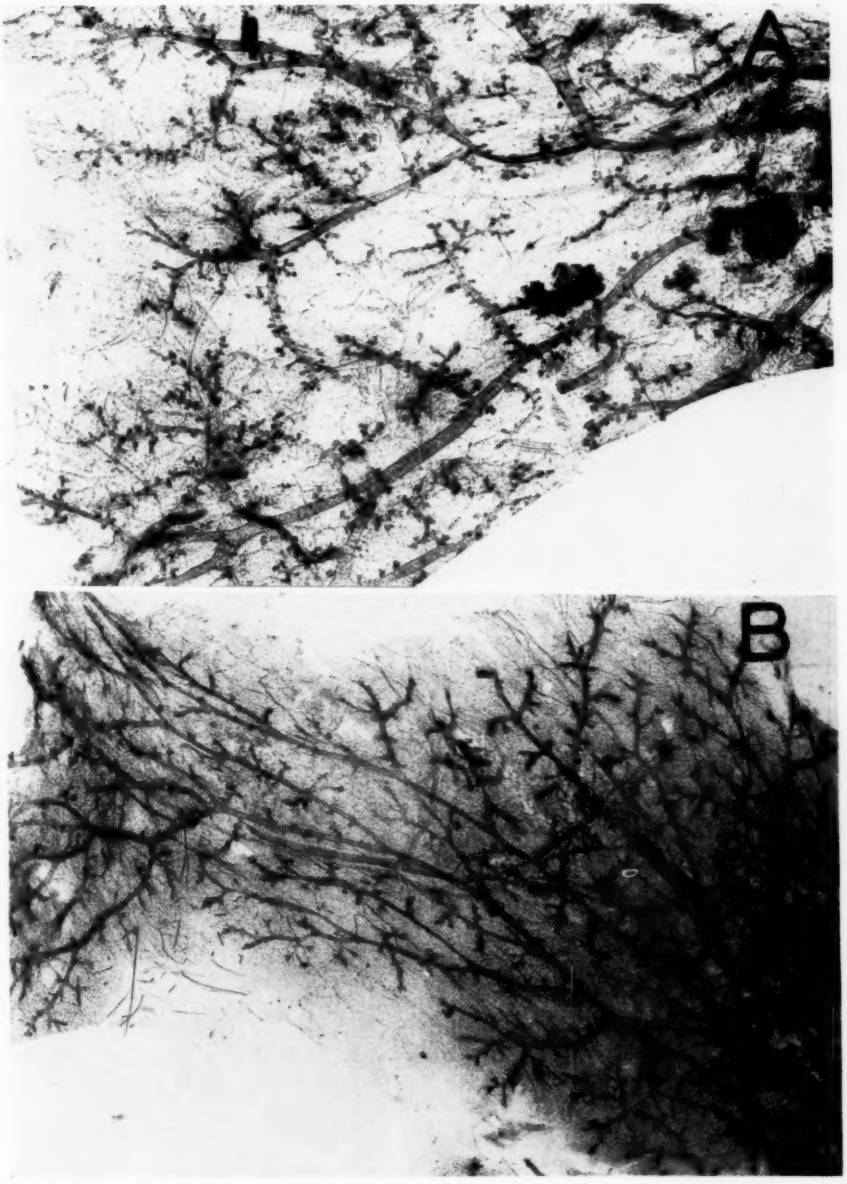

FIGURE 2.—Mammary glands of C3H mice 55 weeks of age. *A*, Control animal. × 10. *B*, Treated animal after 7 weeks of thiourea ingestion. Note beginning involutionary changes in the marginal areas of the gland. × 10.

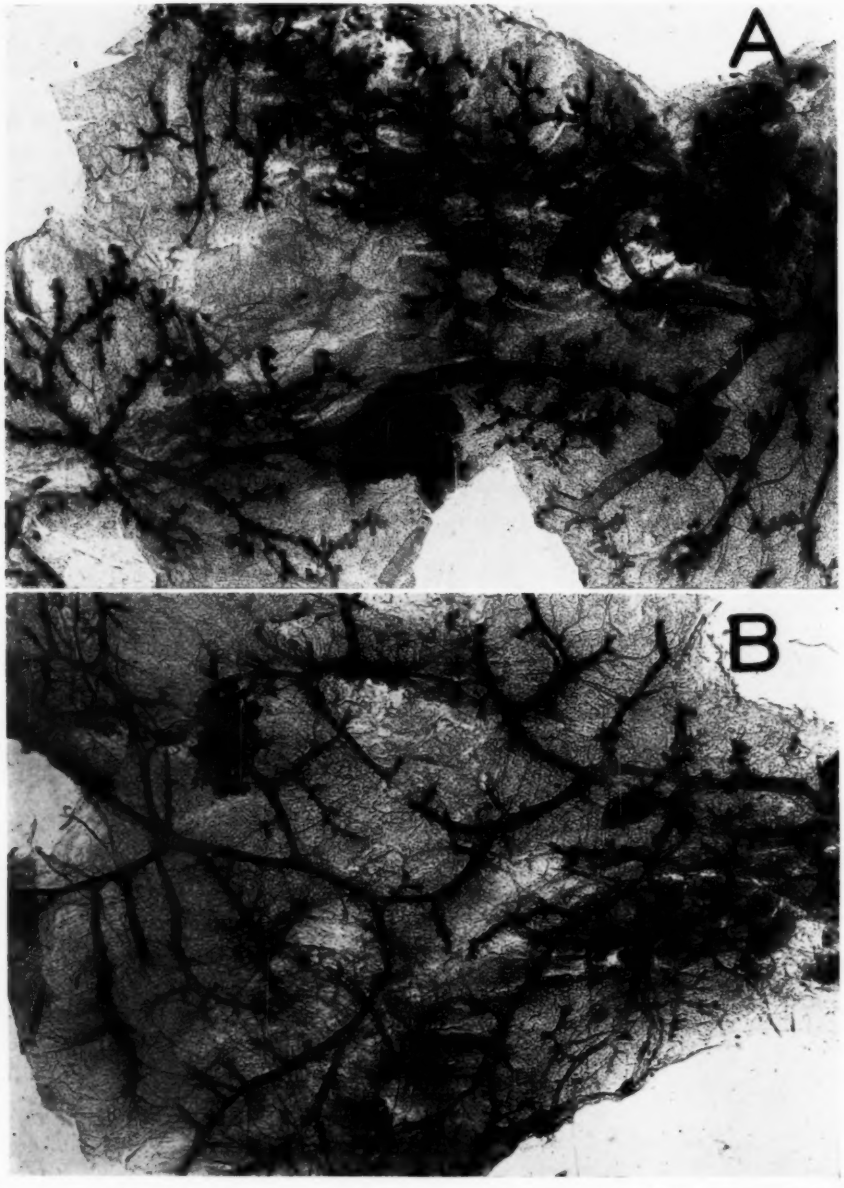

FIGURE 3.—Mammary glands of C3H mice 65 weeks of age. *A*, Control animal. × 10. *B*, Treated animal after 17 weeks of thiourea ingestion. Involutionary changes are present in the peripheral half of the gland. × 10.

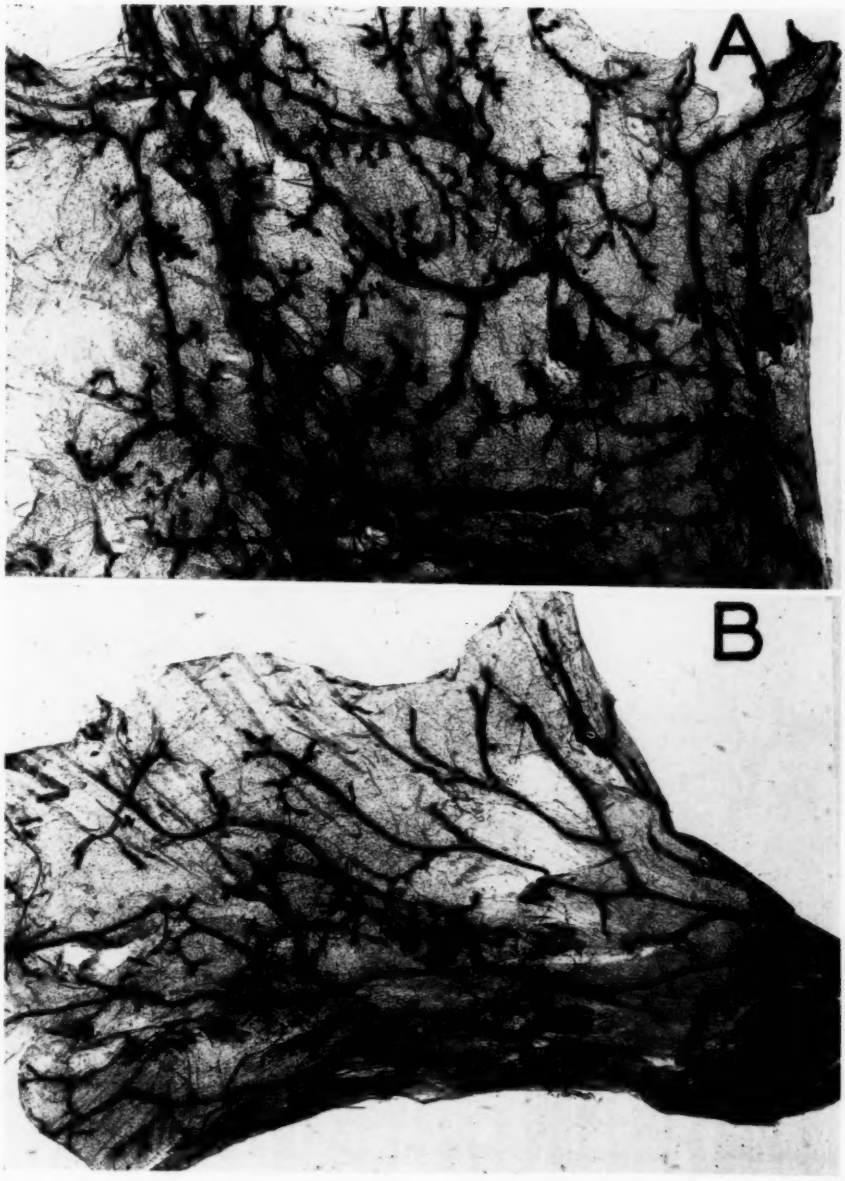

FIGURE 4.—Mammary glands of C3H mice 72 weeks of age. *A*, Control animal. ×10. *B*, Treated animal after 24 weeks of thiourea ingestion. Involution is present in almost all areas of gland. ×10.

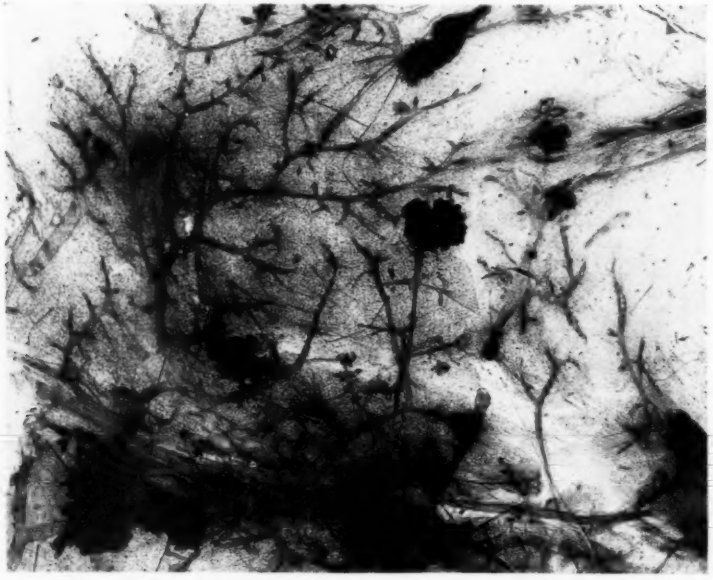

FIGURE 5.—Mammary gland of C3H mouse 92 weeks old after 44 weeks of thiourea ingestion. Pronounced atrophic changes are present throughout the gland. ×10.

FIGURE 6.—Mammary gland of C3H mouse 111 weeks old and after 63 weeks of thiourea ingestion. A very rudimentary duct system remains. ×10.

since virgin C3H females develop tumors before they reach this age if they have the milk factor.

Hyperplastic nodules were present in both the treated and the control animals. The number of animals was not sufficient to permit a comparison between the number of hyperplastic nodules and the length of time the animals were treated with thiourea.

Mammary Tumors

The treatment of adult C3H virgin female mice with thiourea, started when they were 11 months of age, did not reduce the incidence of mammary tumors. Table 3 indicates that in the control group 20 of 23 mice developed tumors, and that in the thiourea-fed group 20 of 21 developed tumors. Three animals in the control group and one in the treated group died without tumors.

Although there was no difference in tumor incidence between the two groups, the treated group did show a delay in the time of appearance of tumors. More tumors developed in the control group during the first half of the experiment than developed in the thiourea-treated group. The average age of the control animals at the appearance of the first palpable tumor was 56.7, and for the treated animals 64.2 weeks (table 4). The average length of time from the beginning of treatment until tumors appeared was 8.7 weeks for the controls, and 16.2 for those receiving thiourea. This difference is statistically significant, having a probability of $0.01 < P < 0.02$. Nine animals of the original group of fifty-three were not included in the data in tables 3 and 4.

TABLE 3.—*Incidence of tumors in C3H mice after ingestion of thiourea and in their controls*

[Numerator=Number of animals developing tumor
Denominator=Number of animals dying without tumor]

Group	Number	Age of mice in months											Total
		11	12	13	14	15	16	17	18	19	20	21	
Control	23	0/0	8/0	5/0	0/0	2/0	3/0	1/1	1/0	0/1	0/1	0/0	20/3
Treated	21	0/0	6/0	2/0	1/0	1/0	2/0	3/0	1/0	1/1	2/0	1/0	20/1

TABLE 4.—*Time of appearance of spontaneous tumors in C3H mice ingesting thiourea and in their controls*

Group	Total mice	Mice developing tumor	Nontumorous mice	Average age of animals to appearance of first tumor	Average time after treatment started to appearance of first tumor
	Number	*Number*	*Number*	*Weeks*	*Weeks*
Thiourea-treated	21	20	1	64.2	16.2
Control	23	20	3	56.7	8.7

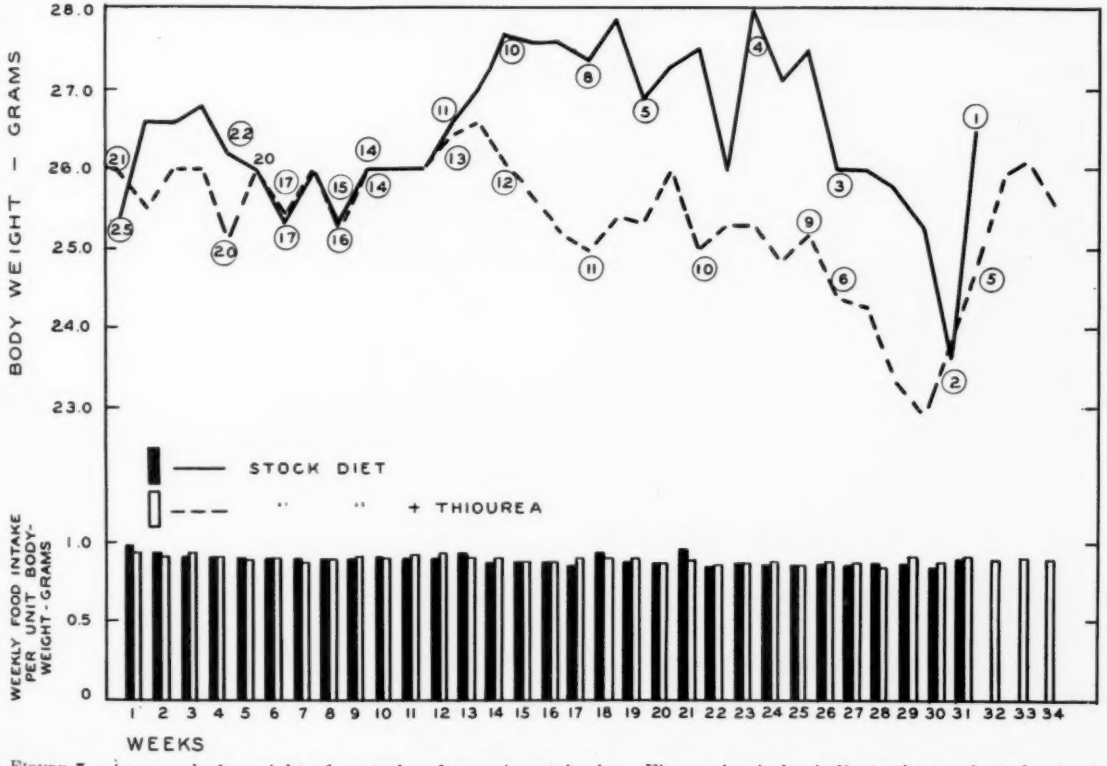

FIGURE 7.—Average body weight of control and experimental mice. Figures in circles indicate the number of animals used in obtaining each point on the curve. Average grams of food consumed by control and experimental animals expressed as grams of food per gram body weight.

There was no evidence that the treatment had any effect on the mammary tumors either grossly or histologically. No studies were made of tumor growth because the tumor-bearing animals were killed 2–4 weeks after the appearance of a tumor to provide suitable material for histologic studies of the adrenal.

Body Weight and Food Intake

The average body weight and food intake for control stock diet mice and mice ingesting thiourea incorporated in the stock diet is given in figure 7. The body weights for the first 13 weeks of the experiment are essentially parallel. The level of thiourea ingestion during this period was 0.25 percent. After 13 weeks, the level of thiourea in the diet was raised to 0.375 percent. The treated mice then showed an average decrease in body weight of 2–3 gm. below the control animals. The food intake expressed as grams of food per week per gram of body weight was essentially the same for both groups.

DISCUSSION

The animals used in this experiment had passed the average age at which spontaneous mammary tumors usually arise in this strain. These old "tumor-free" animals were used because it was thought that any effects of chemical thyroidectomy, especially its influence on mammary-tumor development, would be manifested more quickly in them. Since tumors of microscopic size were undoubtedly present in some animals at the start of the experiment, the delay in the appearance of mammary tumors shown by the thiourea-treated group emphasized the need for further study. We, therefore, set up experiments designed to test the effect of long-continued ingestion of goitrogenic compounds in female mice beginning soon after weaning. The results of these experiments will be reported later.

The ingestion of thiourea by mice in this experiment produced the expected thyroid hypertrophy. Concomitantly, a disturbance of other endocrine organs was indicated by irregularity or absence of estrus in many animals and by the frequent failure to find histologic evidence of cyclic changes in the uterus.[3] Extensive changes were observed in the ovaries, characterized by the presence of many involuting corpora lutea, and few and small follicles with most of the ova degenerate. A progressive involuntionary type of change was noted in the mammary glands. If a delicate balance exists between the thyroid and pituitary, as suggested by Marine (*10*), Means (*11*), Uotila (*12*), Salter (*13*), and others, then it would seem that decreased secretions of the thyroid gland brought about by thiourea ingestion would tend to stimulate thyrotrophic secretion by the pituitary resulting in thyroid hypertrophy. MacKenzie and MacKenzie (*14*) and Astwood et al. (*15*) found that the usual goitrous condition of the thyroid will not develop in hypophysectomized animals ingesting compounds of the thiourea type. The latter observations furnish evidence that the hypertrophy of the thyroid during thiourea ingestion is mediated through the pituitary. In our experiment, the prolonged ingestion of thiourea would appear to have altered not only the thyrotrophic but also other secretions of the pituitary especially the gonadotrophic. This would seem to be a logical explanation for the changes in mammary-gland structure and delay in the appearance of spontaneous mammary tumors in treated mice.

It is believed that the numerous hyperplastic areas which appear in the mammary glands of this strain had already developed by the time the animals were started on the thiourea treatment. Since some such areas were still present after prolonged treatment, ingestion of thiourea did not cause their disappearance. An insufficient number of glands was examined to tell whether or not fewer such areas were present in the treated than in the control animals. It is suggested, however, that the ingestion of an antithyroid compound for long periods, starting prior to the development of the hyperplastic areas, may largely prevent their occurrence. If these hyperplastic areas are the precursors of spontaneous mammary tumors in the mouse, then factors which inhibit their development would also be expected to inhibit mammary tumor formation.

SUMMARY AND CONCLUSIONS

Virgin strain C3H mice, 11 months of age, without palpable spontaneous mammary tumors were divided at random into two groups. One group was placed on a stock diet of natural foodstuffs to which was added 0.25 percent thiourea. After 13 weeks, the amount of thiourea was increased to 0.375 percent. The animals ingested thiourea until mammary tumors developed, or from a minimum of 2 to a maximum of 63 weeks. The control group was maintained on the stock diet throughout.

The incidence of spontaneous mammary tumors was essentially the same in both groups. The appearance of the tumors was delayed in the thiourea-treated group. The average time till tumors appeared was 16.2 weeks in the treated group and 8.7 in the control, a statistically significant difference.

[3] Dalton, A. J., Dubnik, C. S., and Morris, H. P.: Histologic and cytologic changes in the organs of C3H mice ingesting thiourea for long intervals. (To be published in J. Nat. Cancer Inst.)

Estrus cycles were less regular, infrequent, or absent in the treated group when compared with controls of similar age. Whole mounts of mammary glands of treated mice showed considerable atrophy, which became progressively more pronounced in the animals ingesting thiourea for the longer intervals. Hyperplastic nodules of the mammary gland, however, were present in all thiourea-treated animals.

REFERENCES

(1) GARDNER, W. U.: Mammary growth in male mice fed desiccated thyroid. Endocrinology 31: 124–127 (1942).

(2) MIXNER, J. P., and TURNER, C. W.: The mammogenic hormones of the anterior pituitary. II. The lobule alveolar growth factor. Univ. Missouri Agr. Exper. Sta. Research Bull. 378 (1943).

(3) KENNEDY, T. H., and PURVES, H. D.: Studies on experimental goitre. I. The effect of *Brassica* seed diets on rats. Brit. J. Exper. Path. 22: 241–244 (1941).

(4) GRIESBACH, W. E.: Studies on experimental goitre. II. Changes in the anterior pituitary of the rat produced by a *Brassica* seed diet. Brit. J. Exper. Path. 22: 245–249 (1941).

(5) DALTON, A. J. MORRIS, H. P., and DUBNIK, C. S.: Changes in the organs of female C3H mice receiving thiourea. P. Nat. Cancer Inst. 5: 451–454 (1945).

(6) BAUMANN, E. J., and MARINE, D.: Involution of the adrenal cortex in rats fed with thiouracil. Endocrinology 36: 400-405 (1945).

(7) TURNER, C. W., and GOMEZ, E. T.: The normal development of the mammary gland of the male and female albino mouse. Missouri Agr. Exper. Sta. Research Bull. 182 (1933).

(8) MCCLUNG, C. E., ed.: Handbook of microscopical technique for workers in animal and plant tissues. Ed. 2, 698 pp. Paul B. Hoeber, Inc., New York (1937).

(9) HUSEBY, R. A., and BITTNER, J. J.: A comparative morphological study of the mammary glands with reference to the known factors influencing the development of mammary carcinoma in mice. Cancer Research 6: 240–255 (1946).

(10) MARINE, D.: The pathogenesis and prevention of simple or endemic goiter. J. A. M. A. 104: 2334 (1935).

(11) MEANS, J. H.: Diseases of the thyroid gland. New England J. Med. 221: 820 (1929).

(12) UOTILA, U. U.: The regulation of thyrotropic function by thyroxin after pituitary stalk section. Endocrinology 26: 129–135 (1940).

(13) SALTER, W. T.: Endocrine function of iodine. Harvard Univ. Press, Cambridge, Mass. (1940).

(14) MACKENZIE, G. G., and MACKENZIE, J. B.: Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. Endocrinology 32: 185–209 (1943).

(15) ASTWOOD, E. B., SULLIVAN, J., BISSELL, A., and TYSLOWITZ, R.: Action of certain sulfonamides and of thiourea upon the function of the thyroid glands of the rat. Endocrinology 32: 210–225 (1943).

RESPONSE OF CERTAIN MOUSE TUMORS TO BIS (β-CHLOROETHYL) SULFIDE (MUSTARD GAS)[1,2]

By ALLAN D. BASS, *professor of pharmacology*, and MARION L. H. FREEMAN, *research assistant, Department of Pharmacology, Syracuse University College of Medicine*[3]

INTRODUCTION

It has been shown by Krumbhaar and Krumbhaar (*1*) and by Pappenheimer and Vance (*2*) that bis (β-chloroethyl) sulfide (mustard gas) produces specific leukocytic changes in the blood of both experimental animals and human beings. These observations were made during investigations on poison gases during the first World War. Since these studies indicated that bis (β-chloroethyl) sulfide was more effective in reducing the number of circulating lymphocytes than it was in altering other cellular components of blood, it seemed to us that this fact was worth studying in relation to lymphomas. Certain mouse lymphomas, 6C3HED and lymphoid leukemia P1534, were selected for this purpose.

No attempt is made in this investigation to elaborate on the mechanism by which bis (β-chloroethyl) sulfide produces its "leukolytic" effects. A summary of the more recent concepts is given by Gilman and Phillips (*3*).

Since the tissue changes produced by bis (β-chloroethyl) sulfide were previously described, no attempt is made to discuss pathology other than that related to the specific tumors studied.

In addition to the lymphomas included in this investigation, certain mouse tumors of other origins were studied. These are included for the purpose of contrasting the effect of bis (β-chloroethyl) sulfide on lymphoid tumors with a selected variety of nonlymphoid malignant growths.

MATERIALS AND METHODS

Mice bearing transplanted tumors of selected types were obtained from Jackson Memorial Laboratory at Bar Harbor, Maine, and the laboratory of Dr. William U. Gardner, Yale Medical School, New Haven, Conn. Young adult mice of both sexes suitable for growing transplants of these tumors were obtained from Jackson Memorial Laboratory. The bis (β-chloroethyl) sulfide was supplied by the Edgewood Arsenal, Harford County, Md.

Synthetic folic acid, supplied through the courtesy of Lederle Laboratories, was dissolved in phosphate buffer and given intraperitoneally in daily doses ranging from 5 to 100 micrograms. This was used both alone and in combination with bis (β-chloroethyl) sulfide.

The tumor transplantations were made under clean but not aseptic conditions. A small amount of tumor was implanted subcutaneously into the right axillary region with a 16-gage needle. When the tumors became readily palpable (usually about the fifth day), daily records were kept both of the length and width of the tumors and of the weight of the animals.

Treatment was instituted at approximately 7 days after transplantation. Bis (β-chloroethyl) sulfide was dissolved in propylene glycol to make a 0.076-percent solution and was administered intraperitoneally in sublethal doses of from 3 to 6 mg. per kilogram at intervals of 48 hours or longer. A loss of weight frequently observed during therapy suggested the possibility that tumor regression might have resulted from malnutrition. In order to discount this possibility, C3H mice bearing large 6C3HED tumors were fed one-half the average ad libitum food intake. Although these animals showed a weight loss comparable with that seen after administration of bis (β-chloroethyl) sulfide, no regression of the tumor or inhibition of tumor growth resulted.

To demonstrate the effectiveness of therapy in reducing the thickness of the lymphoid tumors the following procedure was used: When animals approached the end of life expectancy, selected treated and control animals, which at the beginning of treatment were approximately the same weight and had tumors of comparable size, were killed. The tumor and adjacent tissues were removed in toto. These tissues included the skin on the one side and the anterior thoracic and abdominal walls on the other. After fixation in 10 percent neutral formalin, a section was cut from the thickest portion of each specimen still retaining the adjacent skin and the abdominal and thoracic walls. After these sections had been photographed, they were prepared for histologic study.

RESULTS

Table 1 shows the effect of bis (β-chloroethyl) sulfide upon certain experimental tumors. Tumors of lymphoid origin showed more regression or inhibition of growth than did tumors of nonlymphoid origin. In the tumors of lymphoid origin, a rather definite and apparently specific change took place. Of the lymphoma 6C3HED, 61 percent showed regression and an additional 9 percent grew less rapidly than did comparable

[1] Received for publication November 13, 1946.
[2] These investigations have been supported by a grant-in-aid from the National Cancer Institute and by a grant from the Hendricks Fund.
[3] The authors wish to express their appreciation to Dr. J. Howard Ferguson, of the Department of Pathology, Syracuse University College of Medicine, for conducting the histologic studies reported in this paper.

TABLE 1.—*Response of certain tumors to bis(β-chloroethyl) sulfide injected intraperitoneally*

Mouse strain	Tumor	Mice treated	Controls	Dose per kilogram			Tumor response					
							Treated mice			Controls		
				Initial	Subsequent	Average doses given	Regression [1]	Inhibition of growth [2]	None	Regression [1]	Inhibition of growth [2]	Normal tumor development
		Number	*Number*	*Mg.*	*Mg.*	*Number*						
C3H	Lymphoma 6C3HED	57	19	3	0	1	34	5	18	0	0	19
		15	2	3	3 or 6	3.75	11	0	4	0	0	2
		15	2	4	4 or 6	3.2	9	1	5	0	0	2
		15	2	5	5 or 6	3.7	6	3	5	0	0	2
Hybrid 6/212	Lymphoid leukemia P1534.	5	5	3	6	2.4	5	0	0	0	0	5
		36	10	3	6	4.02	28	1	7	0	0	10
dba line 1	Myeloid leukemia 1498	55	19	3	6	2.2	0	0	55	0	0	19
dba line 1	Mammary adenocarcinoma dbr B.	15	2	3	3	3.3	1	0	14	0	0	2
		15	1	4	4	3.06	2	0	13	0	0	1
		15	1	5	5	1.8	1	0	14	0	0	1
abc	Spindle-cell carcinoma 15091a.	15	3	3	4	3.6	6	0	9	2	0	1
		15	3	4	4	3.2	6	1	8	2	0	1
		15	3	5	4	2.8	3	0	12	2	0	1
dba line 1	Malignant melanoma S91.	10	10	3	3	2.4	1	0	9	6	0	4

[1] Temporary or permanent reduction in tumor size as determined by length and/or width measurements.
[2] Failure of tumor to grow as large as tumors in control animals.

tumors in untreated animals (fig. 1). In lymphoid tumor P1534, 78-percent regression was observed and an additional 3 percent showed growth inhibition. No significant change was noted in myeloid leukemia 1498, in mammary carcinoma

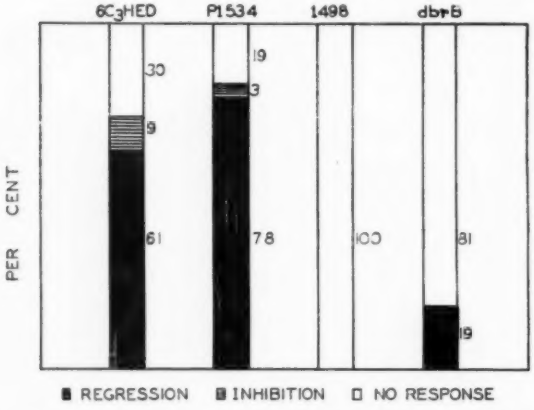

FIGURE 1.—Response of four different mouse tumors to intraperitoneally injected bis (β-chloroethyl) sulfide. The number of animals in each case represents the total number treated, irrespective of dosage schedule.

dbr B, in spindle-cell carcinoma 15091a, or in malignant melanoma S91. Spontaneous regression of tumors 15091a and S91 occurred in many instances. No spontaneous regression or inhibition of growth was observed in the control animals.

A diet restricted to produce weight loss comparable with that seen in animals treated with bis-(β-chloroethyl) sulfide did not affect the growth of lymphoma 6C3HED.

In figure 2 are shown the microscopic characteristics of the 6C3HED tumors. Spontaneous hemorrhage and necrosis occurred in irregularly distributed areas. The tumor cells elsewhere were rather uniform in their growth pattern. In the treated tumors there was, in addition to the areas of spontaneous necrosis, evidence of a diffuse cell necrosis scattered throughout the entire tumor, with all stages of necrobiotic change. Many large phagocytic cells containing debris were present. Viable tumor cells were still present, but necrotic cells predominated. Figure 3 shows the characteristics of the control and treated P1534 tumors. Spontaneous hemorrhage and necrosis are also common in all these tumors. In the treated tumors, degenerative changes are present similar to those seen in the 6C3HED tumors, but in addition, there is rather marked alteration in many individual surviving cells. There is marked pleomorphism with irregularity in the size, shape, and staining characteristics of the viable cells instead of the rather uniform nature of the tumor cells in the control animals.

DISCUSSION

DOSAGE

Although several dosage schedules were used in these studies, no single procedure employed could be predicted to give a uniform response. The variation in response from one animal to another was such that dosage had to be individualized. In general, it was observed that repeated administra-

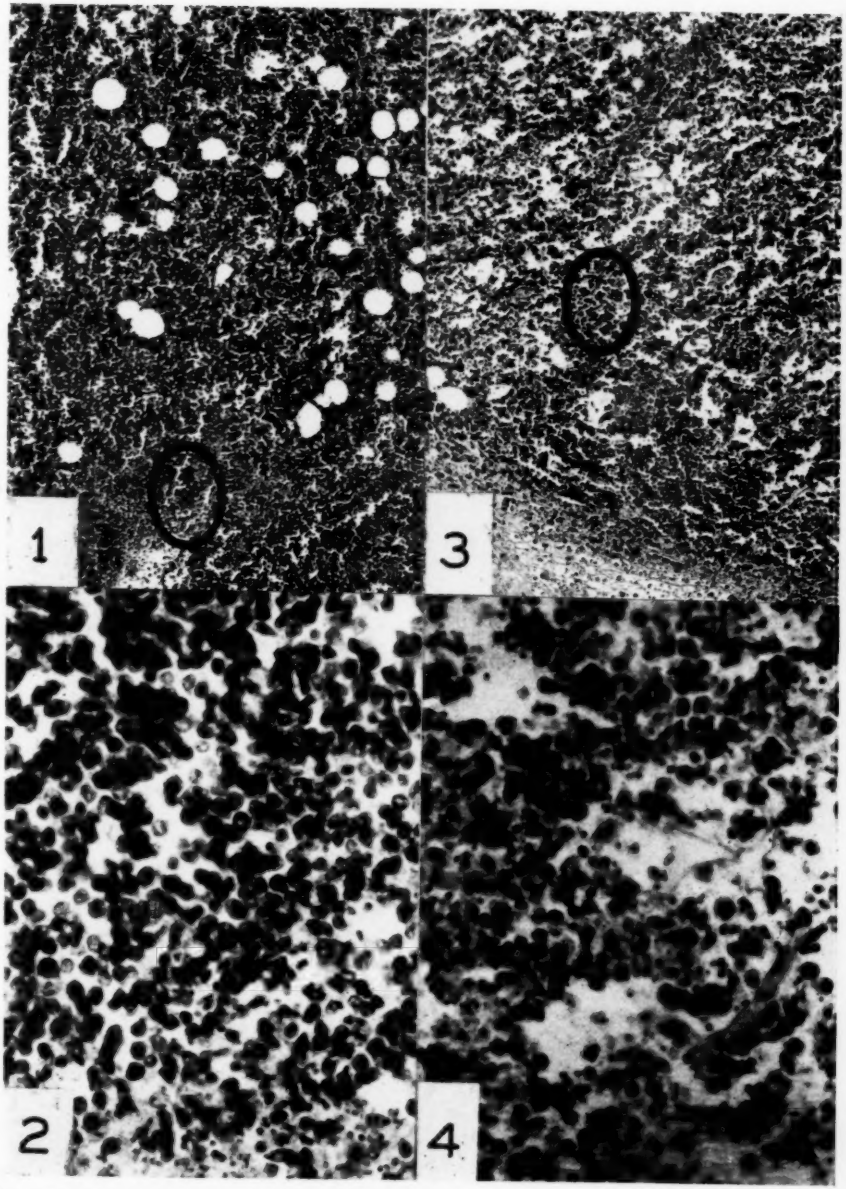

FIGURE 2.—Effect of treatment on lymphoid tumor 6C3HED grown on C3H mouse. 1, Untreated control tumor, × 110. 2, Untreated control tumor, × 420; 3, Tumor from treated animal, × 110; 4, Tumor from treated animal, × 420.

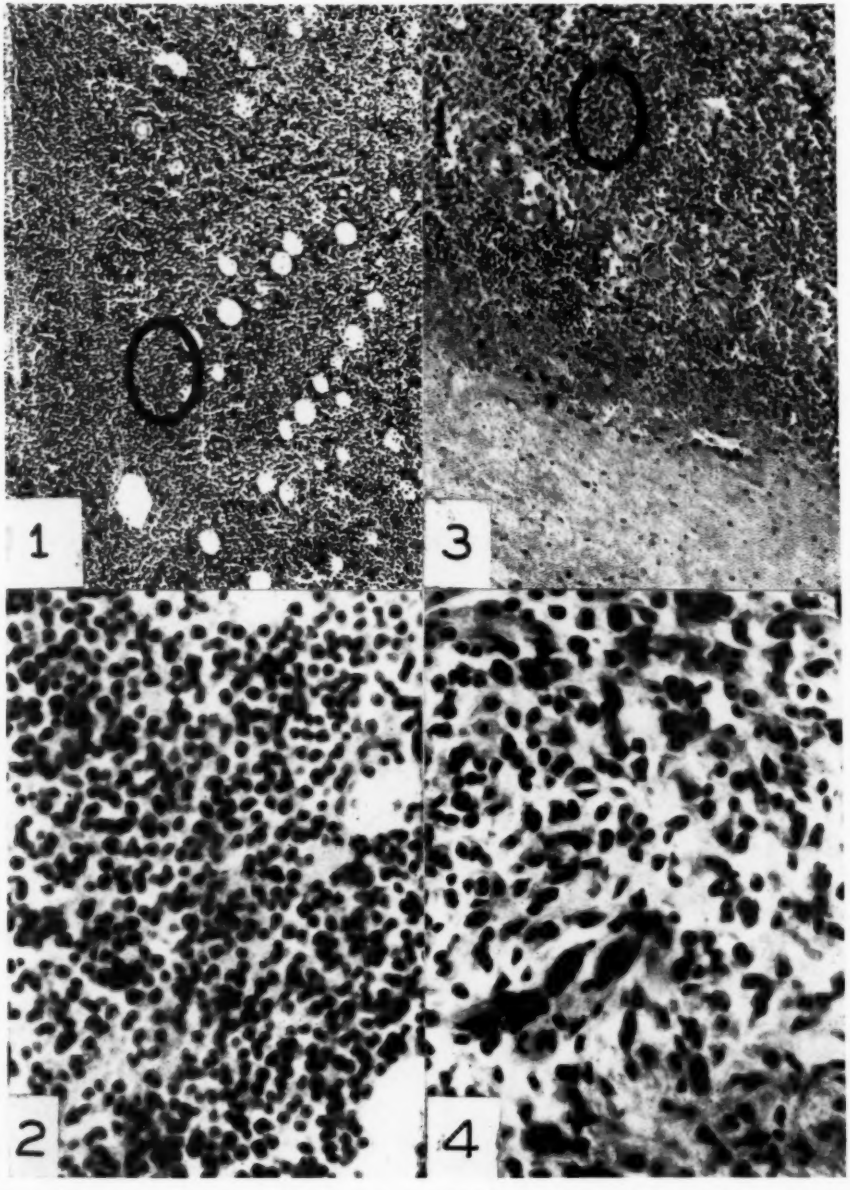

FIGURE 3.—Effect of treatment on tumor P1534 grown in hybrid 6/212. 1, Untreated control tumor, × 110; 2, Untreated control tumor, × 420; 3, Tumor from treated animal, × 110; 4, Tumor from treated animal, × 420.

tions were more efficacious than a single one. The frequency of drug administration was determined more by the condition of the animal (weight loss and diarrhea) than by the tumor response, as the general status of the animal frequently precluded sufficiently frequent bis (β-chloroethyl) sulfide dosage to cause tumor regression.

Tumor Response

The width and length measurements recorded were not entirely satisfactory to show the amount of regression of the lymphoid tumors, since most of these tumors became softer and thinner with therapy. Because of the infiltrating nature of the lymphomas, it was not possible to dissect out and weigh the individual tumors. The results (table 1), however, are based entirely upon the width and length measurements and do not take into account the change in the consistency or the change in thickness. These results, therefore, represent minimum rather than maximum figures. Since we were not able to quantitate changes in the thickness of the tumor, we are not attempting to report the degree of response to therapy, but only the frequency of tumor regression. The degree of response was variable, extending from slight but definite reduction in size to complete disappearance of the tumor.

A reduction in thickness of the tumor was frequently observed and was often more striking than the reduction in the measured diameters. This is shown in figure 4.

Longevity

Although an attempt was made to arrange the dosage schedule to produce the minimum toxic effect on the host and at the same time to cause maximum tumor regression, it was not possible to prolong the life of the tumor-bearing animals. This was true even in those animals where marked regression of the tumor occurred.

Folic Acid

Leuchtenberger et al. (*4*) using sarcoma 180 found that folic acid is a tumor-growth inhibitor. In a few experiments in which we gave folic acid along with bis (β-chloroethyl) sulfide to the animals bearing lymphoma 6C3HED there was apparently less regression of the tumor as well as less toxicity of the bis (β-chloroethyl) sulfide for the host. These investigations are being continued, since the preliminary observations suggest the possibility of establishing a situation in which a therapeutic agent would be less toxic to the host and at the same time equally effective against the tumors.

SUMMARY AND CONCLUSIONS

The effectiveness of bis (β-chloroethyl) sulfide in reducing the size of certain mouse lymphomas is demonstrated.

This effect is apparently rather specific, as four nonlymphomatous tumors showed no response to similar therapy.

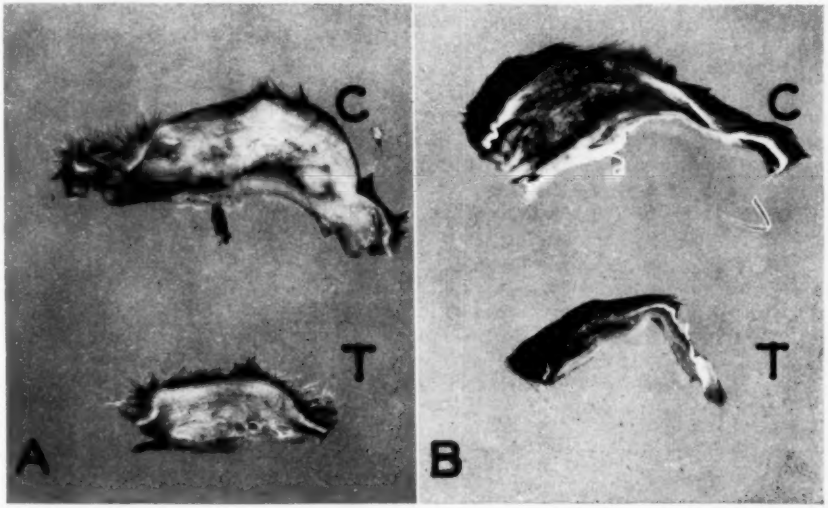

FIGURE 4.—Two lymphoid tumors showing reducing in thickness following therapy with bis (β-chlorethyl) sulfide. *A*, 6C3HED, mouse lymphoma; *B*, P1534, lymphatic leukemia; C, tumor from control animal; and T, tumor from treated animal.

REFERENCES

(*1*) KRUMBHAAR, E. B., and KRUMBHAAR, H. D.: The blood and bone marrow in yellow cross gas (mustard gas) poisoning. J. M. Research 40: 497–507 (1919–20).

(*2*) PAPPENHEIMER, A., and VANCE, M.: Effects of intravenous injection of dichloroethylsulfide in rabbits, with special reference to its leucotoxic action. J. Exper. Med. 31: 71–94 (1920).

(*3*) GILMAN, A., and PHILLIPS, F. S.: The biological actions and therapeutic applications of the β-chloroethyl amines and sulfides. Science 103: 409–415 (1946).

(*4*) LEUCHTENBERGER, C., LEWISOHN, R., LASZLO, D., and LEUCHTENBERGER, R.: "Folic acid" a tumor growth inhibitor. Proc. Soc. Exper. Biol. & Med. 55: 204–205 (1944).

CULTIVATION OF TUMORS IN THE ANTERIOR CHAMBERS OF THE EYES OF GUINEA PIGS[1]

By HELEN M. DYER, *biochemist*, and MARGARET G. KELLY, *assistant biochemist, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

The ability of tumor grafts to grow in heterologous hosts has been considered in the past to be limited to several special sites of transplantation, foremost of which are the developing chick embryo (*1*) and the anterior chamber of the eye (*2*). The literature on this subject has been reviewed by Murphy (*1*), Kerguntul (*3*), Lucké and Schlumberger (*4*), and Greene (*5*). In recent years Greene (*5, 6, 7*) has reported the growth of tumors from various species of animals, including man, in a number of alien hosts. Furthermore, he reports successful growth of tumor grafts in heterologous testicle and muscle in addition to the anterior chamber of the eye. Greene (*6*) has presented evidence in support of a theory that transplants of autonomous tumors (cancers) of heterologous species will invariably grow in the anterior chambers of the eyes of guinea pigs whereas nonautonomous tumors (benign ones) will not grow. In Greene's experience (*6, 8*), the ability of tumors to grow intramuscularly and intratesticularly in heterologous hosts has been aided sometimes by preliminary passage through the eyes of guinea pigs.

Because of Greene's reports it was believed that this technique might make available for study a regular supply of fresh tumor material from a selected cancer of human origin. If, after passage through the eyes of guinea pigs, the human cancer could be carried by intramuscular transplantation in guinea pigs and other experimental animals, sufficient amounts of material might be maintained in the laboratory for protracted study. With this in view, preliminary studies were undertaken to test our use of the method of transfer to the anterior chamber of the eye of the guinea pig.

First-generation transplants to the eyes of guinea pigs were made of 13 different types of histologically and clinically proved cancers from heterologous animal and human hosts, and a third-generation transplant to guinea pigs was made of 1 human tumor.

MATERIALS AND METHODS

The technique employed in these experiments for transfer of tissue to the anterior chamber of the eye was slightly different from that described by Greene (*9*). Transfer of the tumor material was made as soon as possible, and always within 2 hours after removal from the original host. The guinea pig was anesthetized by nembutal injected intraperitoneally. The eye was anesthetized with a 7-percent solution of cocaine hydrochloride and was kept from rolling while an incision was being made by holding the sclera with small, blunt forceps. A sharp knife was used to make an incision horizontally and close to the superior margin of the corneoscleral junction. A small amount of aqueous humor escaped, and under a dissecting microscope a fragment of tumor, measuring approximately 1 mm. in diameter, was inserted into the chamber from a trocar. (The trocar was made of an 18-gage hypodermic needle, by removal of the point, polishing of the cut edge, and insertion of a close-fitting polished plunger.) The eye was examined under the dissecting microscope daily for the first week and once or twice a week thereafter.

Inbred guinea pigs used in this study were between 2 and 36 months (average 10 months) of age. They came from the colony of inbred families of guinea pigs of Dr. W. E. Heston, of this Institute. The market pigs varied from 2 to 12 months of age.

Table 1 records the types of tumors used. The Harding-Passey melanoma of the mouse (group 4) has not been included as one of the clinically proved cancers since there is evidence that metastases of this tumor are rare (*10*). The primary spontaneous mammary adenocarcinomas of C3H mice that were the source of grafts for group 1, metastasize frequently to the lungs, but metastases were not present in the animals that bore the tumors used for transplantation to guinea pigs' eyes. Metastatic material of this same type of tumor was used, however, in group 2. For mouse melanoma S91 (group 3), mouse hepatoma (group 5), mouse fibrosarcoma of the stomach wall (group 6), mouse fibrosarcomas of in vitro origin (groups 7–10) and for Jensen's sarcoma (group 11), the materials used for transfer were from transplanted tumors which metastasize frequently, but no metastases had appeared at the time the hosts were killed for heterologous transplantation.

The fibrosarcomas of groups 8, 9, and 10 arose in C3H mice as a result of intramuscular injections of cultures of fibroblasts (Earle[2] (*4*)). The fibroblasts had been taken originally from a normal C3H mouse and had been grown in vitro in Carrel flasks for varying periods in the presence of 20-

[1] Received for publication November 26, 1946.

[2] A complete series of tumors of this origin had been sent by Dr. Earle to Dr. Greene for studies of their behavior in heterologous transplantations. Meanwhile, we had used strain HGW of the series in our investigation. This sarcoma grew so rapidly and in such a high percentage of the guinea pigs that we thereafter employed other strains of sarcomas of similar origin to check our technique since we were having so little success with the growth of other tumors. Dr. Greene (8) has now published his results with these tumors.

methylcholanthrene in a heterologous medium of horse serum, chick-embryo extract, hen plasma, and physiologic salts. The parent cultures of strains JG, LF, and OA had been treated with methylcholanthrene for 32, 111, and 406 days, respectively, before transfer of the cultures to mice. Strain HGW (group 7) was derived from control cultures (no methylcholanthrene added) of fibroblasts. The various strains had been carried by subinoculation in C3H mice of the Andervont stock for from 49 to 75 tumor generations previous to their transfer to the guinea pigs.

Guinea pigs of family 13 (group 12) received injections of material from twelfth-generation subcutaneous transplants of fibrosarcoma C–2663 which had been induced by 20-methylcholanthrene in a guinea pig of family 13. The pigs in group 13 received transplants of lung metastases of this same fibrosarcoma.

The myxosarcoma of human origin (group 14) was sent to us by Dr. Greene. It was growing for the second generation in the eye of the guinea pig.

The adenocarcinoma from the human colon (group 15), the chest-wall metastasis of a hypernephroma of the kidney of a human (group 16), and the human periosteal sarcoma (group 17) were made available to us through the kindness of Dr. Janvier Whitton Lindsay, pathologist at the Warwick Memorial Clinic for Cancer and Allied Diseases, Washington, D. C. Dr. Lindsay's diagnoses for these three cancers follow:

ADENOCARCINOMA OF COLON, No. 202691

Microscopic examination of biopsy of transverse colon: Paraffin sections show stretches of normal mucosa, interrupted by plaques of incompletely differentiated epithelial structure, the cells and nuclei large and irregular, the process definitely invading the deeper zones. There is extensive and dense fibrosis and marked inflammatory reaction. The condition is evidently malignant and metastasis is recognized in the lymph node reported separately.

Diagnosis: Adenocarcinoma.
Patient (age 35) died 3 months after operation.
Observations at necropsy:

(1) Carcinoma of the transverse colon (operative diagnosis, followed by sections).
(2) Extensive carcinomatosis of the entire abdominal wall, covering the entire peritoneum and extending into the musculature and soft tissues of the right anterior and lateral abdominal wall.
(3) Metastatic carcinoma to the parietal surface of the pleural cavities and the dome of the diaphragm, as well as the external surface of the pericardium.
(4) Metastatic carcinoma of the lower lobe of the left lung.

CHEST-WALL METASTASIS OF HYPERNEPHROMA No. 203954

Gross examination of tumor of chest wall: The tumor from the chest wall appears to be growing directly in the rib, there being a piece of rib attached to each end. The tumor is elongated, measuring 6 x 4 x 4.5 cm. It is encapsulated by periosteum of the rib and thickened shining fibrous tissue. The cut surface presents a firm, shiny, somewhat lobulated, mixed grayish-yellow appearance, with some hemorrhagic areas.

Paraffin sections from tumor of rib show an evidently invading, rather well lobulated structure of large cells generally polygonal in form, and with nuclei of fairly nearly uniform size and morphology, though there are a number of large, irregular forms and mitotic figures. The cytoplasm is abundant as compared with the size of the nuclei and the cells frequently assume a somewhat columnar pattern. Scattered foci of inflammatory reaction, in which plasma cells are predominant, are seen. The picture is typical of hypernephroma.

Diagnosis: Metastatic hypernephroma.
Patient (age 62), alive one year after operation.

PERIOSTEAL SARCOMA No. 206480

Gross examination: Several small particles of "soft tissue" removed from outer surface of the rib, and a particle of bone, apparently degenerated, though no sections are attempted from this part of the material. The "soft tissue" is of rather firm consistency, white in color. Terry section and Terry stain of frozen section show extensive infiltration of the fibrous and muscle tissue of irregular groups and strands of polygonal cells varying considerably in morphology. The process is considered to be malignant and presumably sarcoma. While the source cannot be definitely determined by this examination, it is thought most likely to be bone or periosteum. Frozen section hematoxylin and eosin confirms the foregoing. Paraffin sections later considered to represent bronchogenic carcinoma.

Patient (age 40) died 5 months after operation.

Immediately after transfer to the guinea pigs' eyes, sections from remaining portions of the tumors of human origin (groups 15, 16, and 17) were prepared for histologic examination. No evidence of cell damage could be detected even after a lapse of about 3 hours between removal from the hosts and fixing of the samples for histologic study.[3]

RESULTS

Table 1 summarizes the results. Successful vascularization and growth of transplants were observed in the eyes of 27 guinea pigs. Of these 27 animals, 16 had received transplants of 5 different types of tumor material directly from heterologous hosts; 4 of the guinea pigs showed growth of the human myxosarcoma that had been carried previously by Dr. Greene for 2 generations in guinea pigs' eyes; 2 guinea pigs had received transplants of mouse fibrosarcoma from a first-generation growth in a guinea pig's eye; and 5 of the 27 animals had been inoculated with homologous tumor material from guinea pigs of the same family.

In many of the guinea pigs, vascularization of the eye occurred in the neighborhood of the transplant, and beginning growth was suspected; but the reaction was so transient that those guinea pigs cannot be regarded as having shown positive take-

[3] Dr. Thelma B. Dunn, pathologist, National Cancer Institute, made the histopathologic studies of the material reported in this paper.

(column 7, table 1). In several guinea pigs, there was growth of the transplant without evidence of vascularization. In all but two animals (group 8), however, the tissue-culturelike growth was followed by vascularization and further growth.

Among 66 guinea pigs of groups 1, 9, 11, 14, 15, and 16, in which no growth of the transplants was observed, 11 showed latent vascularization and growth of foreign materials in the inoculated eyes. When these animals were killed, 3 to 7 months after transplantation, bone (identified histologically) was found to have developed in a manner similar to that of successful tumor transplants in the anterior chambers of the eyes. Mesina (*12*) observed osteoid change in guinea pigs' eyes following transplantation of adenocarcinoma of the prostate gland associated with androgen therapy of the animals.

TABLE 1.—*Results of transfer of tumors to the anterior chamber of the guinea pig's eye*

Group No.	Tumor material used as transplant			Guinea pigs receiving anterior-chamber transplants			
	Host	Source	Microscopic diagnosis	Number	Genetic No. of family	Animals showing—	
						Vascularization and questionable growth [1]	Unquestionable growth
						Number	*Number*
1	C3H mouse	Spontaneous primary	Mammary adenocarcinoma	18	13	7	0
2	do	Lung metastases	do	3	Market	1	0
3	dba mouse	Transplanted sc	Melanoma S91	4	13	0	0
				4	2	1	0
				1	32	0	0
				4	Market	1	1
4	C mouse	do	Harding-Passey melanoma	4	32	0	0
				4	Market	0	0
5	C3H mouse	do	Hepatoma (induced)	4	13	0	0
6	do	do	Fibrosarcoma of stomach wall (induced).	3	32	0	0
7	do	Transplanted im	Fibrosarcoma HGW/49A4, A5 (in vitro history).	4	2	1	3
				5	13	0	5
7a	Guinea pig family 2	Transplanted anterior chamber (first).	do	2	2	0	2
8	C3H mouse	Transplanted im	Fibrosarcoma JG/70A1 (in vitro history).	3	32	0	3
				4	Market	[2] 2	1
9	do	do	Fibrosarcoma LF/75A (in vitro history).	3	32	2	1
				4	Market	2	1
10	do	do	Fibrosarcoma OA/52A37 (in vitro history).	4	32	0	[3] 1
11	Osborne-Mendel rat	Transplanted sc	Jensen's sarcoma	4	13	2	0
				6	2	3	0
12	Guinea pig family 13	do	Fibrosarcoma C-2663 (induced)	6	13	1	3
13	do	Lung metastases	do	4	13	1	2
14	Guinea pig from man	Anterior chamber transplant (Greene HC170).	Myxosarcoma from man	4	32	1	2
				4	13	1	0
				2	Market	0	2
15	Man	Primary, of colon	Adenocarcinoma	10	32	4	0
16	do	Chest-wall metastases	Hypernephroma	11	32	0	6
17	do	Primary	Periosteal sarcoma	4	32	0	0
				4	Market	1	0

[1] Under dissecting microscope vascularization was observed, and the transplant appeared to increase slightly in size. This reaction was very transient, and no proof of growth was obtained.
[2] Tissue culture type of growth only, without vascularization of growing transplant.
[3] Transplant became vascularized and grew to one-fifth the diameter of the cornea when regression began. When the guinea pig was killed on the thirty-seventh day, the transplant was lost during dissection of the eye, and no proof of identity was obtained.

With the exception of melanoma S91, which grew in the eye of 1 of 13 guinea pigs inoculated, and the myxosarcoma which had been transferred previously through guinea pigs' eyes for 2 generations, the only tumors that grew in heterologous hosts were the 4 strains of fibrosarcoma that had arisen in mice from intramuscular injections of fibroblast cultures from Carrel flasks and had been carried serially in mice by intramuscular transplantation. The transplants of these fibrosarcomas vascularized within 2 or 3 days, and growth was extensive 9 days after transplantation. Rapid vascularization and growth were observed also with the fibrosarcoma of the guinea pig (groups 12 and 13) but not with the mouse melanoma or the human myxosarcoma, which failed to show definite vascularization and growth until 4 to 6 weeks after transplantation.

In four of the guinea pigs, the inoculated eyes were bulging, and the transplants had attained the diameter of the cornea when regression began. In the other guinea pigs' eyes in which there was growth, the transplants began to regress after growing to one-third to two-thirds of that diameter. When the larger transplants were removed from the eyes for examination, they were found

to be noninfiltrating, discrete, and disk-shaped growths, never larger than 8 x 8 x 1.5 mm.

In the eyes in which the tumors failed to grow, the transplants were observed to remain at the sites of transplantation for 1 to 4 weeks or longer.

Histologic examination of the growing transplant from at least one eye of each group (except group 10) demonstrated the identity of the transplants that grew in the guinea pigs' eyes with the original tumors used for transfer. With three grafts (one each of mouse fibrosarcoma HGW, guinea pig fibrosarcoma C–2663, and the human myxosarcoma), histologic sections of materials removed after vascularization of the transplant had begun to disappear were found to have only remnants of necrotic tumor tissue with no viable cells present. The remaining animals with growing transplants were kept for observation of the fate of the tumors. In every case, the transplants had regressed completely within 16 to 59 days following transplantation.

The fibrosarcoma of the guinea pig (group 12) grew in the eyes of three of six guinea pigs to which material from a subcutaneously transplanted tumor was transferred, and in two of four guinea pigs (group 13) which received transplants of the metastatic fibrosarcoma.

At the time of the successful transfer of the HGW strain of mouse fibrosarcoma from the first to the second generation in the guinea pigs' eyes (group 7a), transfers were also made intramuscularly, by trocar, into the leg muscles of two guinea pigs, three rats, and two rabbits. There was no evidence (by palpation) of growth of the transplants in any of these animals. The muscles were found to be normal in appearance when the animals were killed, the rats after 6 weeks, and the guinea pigs and rabbits after 6 months.

DISCUSSION

Failure to obtain the growth of transplants in the anterior chambers of guinea pigs' eyes with 7 of 14 clinically proved cancers, the transient character of the transplants that did grow, and finally the small amounts of tumor material present at the time of maximum growth of the transplants indicated that for our purposes transplantation to the anterior chamber of the eye did not provide a practical method for the routine growing of human tumor tissue.

It is obvious that the question of the ability of a tumor to grow in the eyes of heterologous species cannot be decided without using a large number of animals. The individual response of the heterologous host used for the transplantation, as well as the nature of the tumor material, appears to influence the fate of the transplant. This is especially evident in the results with melanoma S91, which grew in the eye of 1 of 13 guinea pigs inoculated. If the numbers of guinea pigs used for anterior-chamber transplantations in this study were assumed to be sufficient for conclusive evidence, then, according to Greene's criterion (*6*), the 3 human tumors, the Harding-Passey melanoma of mice of strain C, the induced hepatoma of C3H mice, and Jensen's sarcoma from Osborne-Mendel rats would be considered nonautonomous (benign); while the transplanted melanoma S91 of dba mice, the 4 transplanted fibrosarcomas of mice from Earle's in vitro cultures of fibroblasts, and the human myxosarcoma would be diagnosed as autonomous tumors (cancers). It may be presumed that a larger number and variety of heterologous hosts used for anterior-chamber transfers would result in additional takes. Nevertheless, the use of this criterion in determining that a tumor is benign must be accepted with the same reservations as the use of negative results of a limited series of tests for carcinogenesis as a criterion of noncarcinogenicity of a compound.

Greene (*8*) reported that—

> the tumors induced *in vitro* by Earle have been successfully transplanted to guinea pigs and to mice of foreign strains. The ability to survive and grow in animals of alien species identifies the tumors with the chemically induced and naturally occurring sarcomas, and adds further significance to their mode of origin.

In Greene's study, a high percentage of takes was obtained with anterior-chamber transplants in guinea pigs' eyes with all the strains of these sarcomas except strain OA, which was reported to grow, but wholly in the manner of a tissue culture without vascularization. With the limited number of animals used in the present study for anterior-chamber transplants of strains HGW, JG, LF, and OA of the tumors induced in vitro, a high percentage of takes was obtained with strain HGW, and more than 50 percent with strain JG; transplants of LF grew in two of seven guinea pigs; unfortunately with strain OA, of which growth of the transplant was observed in only one of four guinea pigs, regression had begun on the thirty-seventh day when the guinea pig was killed and the transplant lost during dissection of the eye, thus leaving no histologic proof of growth.

In this series of experiments, the transplanted mouse sarcomas (groups 7–10) that originated from fibroblasts that had been exposed in vitro to heterologous media for long periods were relatively successful in growing in the anterior chambers of the eyes of the heterologous hosts. Whether or not this comparative ease of adaptability was a result of the previous history of the parent cells of the tumor cannot be decided in this limited study. Greene (*8*) observed increases in range of transplantability and in the incidence of takes after residence of tumor material in foreign hosts.

The Jensen sarcoma, which was utilized in group 11 with negative results, was used in one of the earlier investigations of heterologous growth of tumors. In 1913 Murphy (*1*) reported the successful serial transfer of this rat tumor into developing chick embryo. Transfer back to the rat resulted in takes, but all attempts to transplant the tumor into adult chickens failed.

Serial subcutaneous transfer of the fibrosarcoma of the guinea pig (groups 12 and 13) into guinea pigs of the same family does not give a high percentage of takes; although when transplantation is successful, large tumors result, and metastases to the lungs are frequent. It was hoped that passage of this tumor through the eyes of guinea pigs might result in material which would give a higher percentage of takes thereafter in subcutaneous transplantation. The first-generation transplants in the eyes behaved as did the successful heretologous tumor transplants to eyes, regressing in all but one guinea pig before filling the anterior chambers. The guinea pig with the largest tumor in the eye was killed. The tumor, which was readily removed by means of forceps, measured approximately 8 x 8 x 1.5 mm. Since experience [4] had demonstrated the need of large amounts of material for successful subcutaneous transplantation of this tumor, the possibility of increasing the number of subcutaneous takes by passage through guinea pigs' eyes did not warrant further study.

The fact that regressions occurred in most cases before the anterior chambers of the eyes were filled with tumor material would indicate from Greene's experience (*13*) that the guinea pigs had developed immunity to the transplants. No tests were made with transplants to the other eye to investigate the existence of resistance. However, early regressions of tumors after limited periods of growth in heterologous hosts do not seem to be unusual. Lucké and Schlumberger (*4*), in reviewing the literature on the subject of the earlier attempts to grow tumors in the anterior chambers of the eyes of heterologous animals, cited several reports of successful but transient growth. Greene (*8*) reported early regressions of the "*in vitro* induced" mouse tumors growing in guinea pigs' eyes and observed that rapidly growing transplants usually end in regression in the eye. The early regression of tumors transplanted into the muscles and testicles of animals of alien species was also observed by Greene and Murphy (*7*). Taylor and Kynette (*14*) observed that a persisting growth of mouse mammary adenocarcinoma was never obtained in the anterior chambers of rats' eyes. Putnoky (*15*) transferred the Ehrlich mouse carcinoma subcutaneously into adult rats, using a large inoculum of tumor, and obtained rapid growth, but he reported that regressions begin by the tenth day and that transfer must be made at that time. Mesina (*12*) recently reported the failure of anterior-chamber transplants of histologically proved carcinoma from human prostates to survive more than 30 days. These regressions of transplants, without evidence of any deleterious effect other than local damage to the eye of the heterologous host as a result of the transient growth of the tumor material, appear to support the classical thesis that cancers are specific in their virulence for the species in which they arise.

[4] Heston, W. E.: Private communication.

SUMMARY

Transplants of 14 different types of histologically and clinically proved cancers from mouse, rat, guinea pig, and human hosts were transferred to the anterior chambers of guinea pigs' eyes.

Under the conditions of the experiment, growth was not obtained with primary mammary adenocarcinomas of C3H mice or with metastatic material of that tumor, with the transplanted Harding-Passey melanoma of mice of strain C, with a transplanted fibrosarcoma of the stomach of C3H mice, with transplanted Jensen sarcoma of Osborne-Mendel rats, with a primary adenocarcinoma of the human colon, with a chest-wall metastasis of hypernephroma of the human or with a primary human periosteal sarcoma.

Growth was obtained with transplanted melanosarcoma S91 of the mouse, with human myxosarcoma HC170, with transplanted fibrosarcoma C-2663 of the guinea pig, and with four different strains of transplanted fibrosarcomas of mice of strain C3H. The fibrosarcomas arose in C3H mice originally as a result of intramuscular injections of cultures of mouse fibroblasts that had been grown in Carrel flasks in a heterologous medium. Three of the strains (JG, LF, and OA) had been grown in vitro in the presence of 20-methylcholanthrene for varying periods. Methylcholanthrene had not been added to the culture medium of control strain HGW.

Growth of transplants was transient, and the guinea pig hosts, except for the inoculated eyes, appeared to be unaffected by the growth of the cancer material from the alien species.

From these results it appeared that very large numbers of guinea pigs would be required to insure the growth of human-tumor grafts and that extremely small amounts of tumor material could be expected to be available at the time of maximum growth of the transplant in the guinea pigs' eyes.

REFERENCES

(1) Murphy, J. B.: Transplantability of tissues of the embryo of foreign species. Its bearing on questions of tissue specificity and tumor immunity. J. Exper. Med. 17: 482–493 (1913).

(2) Hegner, C. A.: Ueber experimentelle Uebertragung von Tumoren auf das Auge. München. med. Wchnschr. 60: 2722–2924 (1913).

(3) Kerguntul, R.: La greffe des tumeurs malignes entre espéces animales distinctes, ses difficultés et les elements biologiques du problème. Monographie Inst. Pasteur, No. 15, Paris (1933).

(4) Lucké, B., and Schlumberger, H.: The manner of growth of frog carcinoma, studied by direct microscopic examination of living intraocular transplants. J. Exper. Med. 70: 257–268 (1939).

(5) Greene, H. S. N.: Heterologous transplantation of mammalian tumors. I. The transfer of rabbit tumors to alien species. J. Exper. Med. 73: 461–474 (1941).

(6) Greene, H. S. N., and Lund, P. K.: The heterologous transplantation of human cancers. Cancer Research 4: 352–363 (1944).

(7) Greene, H. S. N., and Murphy, E. D.: The heterologous transplantation of mouse and rat tumors. Cancer Research 5: 269–282 (1945).

(8) Greene, H. S. N.: The heterologous transplantation of mouse tumors induced in vitro. Canver Research 6: 396–402 (1946).

(9) Greene, H. S. N., and Saxton, J. A., Jr.: Uterine adenomata in the rabbit. I. Clinical history, pathology and preliminary transplantation experiments. J. Exper. Med. 67: 691–708 (1938).

(10) Harding, H. E., and Passey, R. D.: A transplantable melanoma of the mouse. J. Path. & Bact. 33: 417–427 (1931).

(11) Earle, W. R., and Nettleship, A.: Production of malignancy in vitro. V. Results of injection of cultures in mice. J. Nat. Cancer Inst. 4: 213–227 (1943).

(12) Mesina, M. H.: Heterologous transplants of human carcinoma of prostate. J. Urol. 53: 257–262 (1945).

(13) Greene, H. S. N.: The participation of the anterior chamber of the eye in resistance phenomena related to tumor growth. Cancer Research 2: 669–674 (1942).

(14) Taylor, A., and Kynette, A.: Evidence for a cancer virus using intraocular implants of mouse tumor in rats. Univ. Texas Pub. 4507: 32–42 (1945).

(15) Putnoky, J.: Über die heteroplastische Transplantation des Ehrlichschen überimpfbaren Mäusecarcinoms. Ztschr. f. Krebsforsch. 32: 520–525 (1930).